

SALT AND WATER BALANCE IN THE SPIDER  
*PORRHOTHELE ANTIPODIANA* (MYGALOMORPHA: DIPLURIDAE)

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## ABSTRACT

Salt and Water Balance in the Spider *Porrhothele antipodiana*  
(Mygalomorpha: Dipluridae)

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Total ion and water balance of the mygalomorph spider *Porrhothele antipodiana* under conditions of feeding and starvation, hydration and dehydration, and experimental salt loading was studied. Transpirational waterloss was comparable to that of xeric arthropods, and respiratory waterloss was reduced by the action of spiracular valves. Waterloss associated with the silk was small, but elevated activity during web construction increased total waterloss. Excretory waterloss occurred in the urine and the coxal fluid. Urine was produced at a rate of  $5-7 \mu\text{l day}^{-1}$  by hydrated starved animals, but increased markedly following feeding. Coxal excretion was normally limited to periods of feeding and fluid was produced at a rate of  $72 \mu\text{l hr}^{-1}\text{gm animal}^{-1}$ . It is transported forward, via cuticular grooves on the ventral surface of the cephalothorax, to the mouth. Seventy percent of the fluid is reingested, implicating it in the feeding process.

Dehydration results in a cessation of urine production by starved animals and a marked reduction in the volume excreted following feeding. The rate of coxal excretion during feeding is inversely related to the hydration state of the animals.

The coxal fluid is generally somewhat hypo-osmotic to the haemolymph and is rich in Na ions. It thus represents an important vehicle for the elimination of Na. In contrast the anal system (Malpighian tubules, stercoral pocket etc.) produces a fluid of low Na/K ratio and is the major route for K elimination.

It is demonstrated that for the animal to remain in salt and water balance a source of free water is required in addition to the prey. Reductions in rate of urine and coxal fluid production did enable the spiders to achieve a net gain of water from the prey, but the degree of concentration of these fluids was inadequate to excrete all of the ions gained during feeding.



I. SALT AND WATER BALANCE IN HYDRATED *PORRHOTHELE ANTIPODIANA*.

## I.1 INTRODUCTION

The most physiologically demanding feature of the terrestrial environment is, perhaps, the shortage of water. This is particularly true for the small terrestrial arthropods, which have a large surface to volume ratio and so are highly susceptible to waterloss. Thus survival in the terrestrial environment demands an osmoregulatory system that is efficient in conserving water, but equally a system that possesses the flexibility to adapt to the short term perturbations in ion and water balance associated with rapid changes in environmental conditions or with feeding. Among invertebrates only representatives of the arachnida and the insecta have achieved a degree of terrestriality which suggests that they may possess such an osmoregulatory system.

Of the terrestrial arachnids one of the most successful groups and certainly the most well known to man, is the spiders. This highly varied group of carnivorous, predominantly insectivorous, organisms with some thirty thousand species (Kaestner, 1968) is represented in large numbers throughout the terrestrial environment. Remarkably little is known of their osmoregulatory physiology.

The little information available which relates to the osmoregulatory physiology of the spiders concerns, in the main, mechanisms for reducing transpirational losses. Spiders are known to have a low rate of total evaporative waterloss, equivalent to that of insects of comparable size (Davies and Edney, 1952; Cloudsley-Thompson, 1957; Stewart and Martin, 1970; Seymour and Vinegar, 1973; Humphreys, 1975), and Hadley (1978, 1981) has demonstrated that spiders, like the insects, possess a fine layer of epicuticular waxes which are thought to confer low permeability. Reductions in losses across the respiratory surface by the action of spiracular valves present at the openings of the booklungs of spiders have also been demonstrated (Davies and Edney, 1952; Cloudsley-Thompson, 1957; Stewart and Martin, 1970).

However, the significance of excretory waterloss and the mechanisms by which it is reduced is poorly understood, and limited to considerations of nitrogenous excretion. Several authors have shown that guanine is the major nitrogenous excretory product of the spiders (Vajropala, 1935; Schmidt *et al*,

1955; Atkinson and Chorlton, 1956; Anderson, 1956<sup>66</sup>) and Anderson (1966) have noted the advantages, in terms of water conservation, that guanine confers because of its low solubility.

Equally poorly understood are the means by which spiders replenish losses of water. Stewart and Martin (1970) reported that the tarantula, *Dugesia hentzi*, drank regularly and replaced blood volume lost through desiccation and bleeding, by drinking. However, it is generally held that the spiders obtain their water requirements from their prey.

Whereas aspects of the osmoregulation of spiders pertaining to the water balance have received cursory inspection, salt balance and ion excretion in the spiders has been completely neglected. As far as I am aware there have not been any reports dealing with the salt balance and routes of salt excretion in the spiders. Indeed our knowledge of the excretory physiology of the spiders is so limited that there is room for dispute as to which are in fact the excretory organs of spiders.

In the spiders there are three organs which, at least superficially, are structurally similar to excretory organs of other arthropods, the Malpighian tubules, the stercoral pocket and the coxal glands. The Malpighian tubules of spiders resemble those of insects in that they are blind tubular structures branching from the gut in the vicinity of the hindgut. They are, however, endodermal in origin, rather than ectodermal as in the insects, and unlike the tubules of the insects which lie free in the haemolymph, those of the spiders branch further and ramify throughout the compact tissue mass, consisting of the abdominal diverticula and storage material, which fills most of the abdomen of spiders. The stercoral pocket is a sac like diverticulum of the hindgut, and its position in the body is reminiscent of the rectum of the insects. The coxal glands are located in the cephalothorax of all spiders, although there appears to be a trend toward the reduction in complexity of the coxal glands and a concentration of the remaining elements in the anterior region of the cephalothorax (Buxton, 1913). These glands are representatives of the diverse group of coelomoduct derived organs which were primitively excretory (Goodrich, 1945; Clarke, 1979), but which have been subsequently extensively modified in the arthropods and now may function as either salivary, excretory or reproductive organs (Goodrich, 1945).

Millot (1949) has proposed that because of their developmental morphology and physiological activity the Malpighian tubules are the primary organs of excretion in the spiders. Furthermore, Millot argued that the coxal glands are no longer excretory organs, citing their reduction in more advanced spiders as evidence of their obsolescence, their excretory function having been taken over by the Malpighian tubules. Much support for Millot's proposal has been drawn from the demonstration that the Malpighian tubules and rectum are the major excretory organs in the insects (see Maddrell, 1971; Wall and Oschman, 1975; Edney, 1977 for references). However, Millot's evidence for physiological activity is tenuous, limited to the demonstration that the tubules will concentrate dyes.

Clearly many questions concerning the osmoregulatory physiology of the spiders remain unanswered; what is the significance of the prey water to spiders?, how do spiders excrete excess ions ingested with the meal?, do the coxal glands serve an excretory function?, what are the effects of dehydration, starvation and feeding on the composition of the haemolymph and the functioning of the excretory systems?, can spiders produce a concentrated urine?, and so on. This study is an attempt to provide answers to some of these questions by considering the ion and water balance of a single generalised spider, *Porrhothele antipodiana* (Walkenaer, 1837).

*P. antipodiana* is a representative of the mygalomorph spiders, a group which includes the tarantulas, trap door and purse web spiders. They are ancestral to, and more generalised than the true spiders, or araneomorphs (Gerstch, 1979), the obvious difference between the two groups being the presence of two pairs of booklungs and paraxial chelicerae in the mygalomorphs (Forster and Wilton, 1968).

*P. antipodiana* is found throughout the North Island of New Zealand, and in the South Island, north of Balclutha on the east coast and Hokitika on the west coast. It spins extensive funnel like webs beneath stones, logs and loose bark of trees. Some merit was seen in choosing a representative of the more generalised mygalomorphs for this first detailed study of the osmoregulatory and excretory physiology of a spider. In addition *P. antipodiana* has the advantages of abundance, ease of collection and large size.

The text is divided into four sections, each with an introduction and

a summary of the main findings. The first section, presented here, introduces in more detail the excretory system of *P. antipodiana*, and spiders in general, and considers the total salt and water balance of hydrated animals, both starved and feeding. Experiments showing the significance of prey and drinking water are reported and excretory losses determined. The possible routes of salt excretion are also investigated, and a complete salt balance for feeding animals presented. The following three sections extend from results obtained in the first section. Interestingly an important avenue for the elimination of salts by feeding *P. antipodiana* involves excretion into the prey during feeding. In Section II this is investigated in more detail and shown to involve coxal excretion. Coxal and anal excretion under conditions of salt loading are also investigated. The third section deals with the transpirational components of waterloss and considers the effects of dehydration on the composition of the haemolymph. The final section (IV) investigates the effects of water deprivation and dehydration on the functioning of the excretory systems of *P. antipodiana* and the resultant ion and water balances of dehydrated spiders are presented.

## I.2 METHODS AND MATERIALS

Adult female *P. antipodiana* were collected from Third Bay and Whalers Bay on the Kaikoura Peninsula, New Zealand. They were maintained in the laboratory in individual clear plastic containers (14×11×6 cm) at  $20 \pm 2^\circ\text{C}$  and under natural light conditions. A continual supply of water was available to the spiders, and four or five large cockroach nymphs (*Periplaneta americana*) were provided each week as food. Spiders were maintained under these conditions for at least a week before use in experiments.

During experiments spiders were placed in individual acid cleaned glass dishes (8 cm diameter, 6.5 cm deep) all sides of which were lined with Whatman 542 filter paper. When required, water was provided by placing absorbent cotton wool rolls, soaked in tapwater, in small plastic troughs on the bottom of the dishes. Cockroaches from the same culture used for the general maintenance of spiders were used as food in ion balance and other experiments where spiders were fed. This cockroach culture was maintained on a standard diet.

Spiders were transferred to clean containers with fresh filter paper and water every 24 hours. To avoid contamination of the filter paper the glass dishes were washed in 50%  $\text{HNO}_3$  for 24 hours, rinsed three times in distilled water, soaked overnight in distilled water and then oven dried. At all times filter paper and clean dishes were handled with clean plastic gloves (Med x Disposable Gloves, Fabri Cell Products, Auckland).

### I.2.1 Determination of Rate of Anal Urine Production and Cation Excretion

The urine and faeces excreted by *P. antipodiana* during experiments adhered to and dried on the filter paper lining the experimental chambers. When dry the urine was clearly visible under ultra violet light and the volume excreted was estimated from the diameter of the urine spots using a calibration curve which related urine volume to spot diameter. The calibration curve was constructed by spotting standard volumes of fluid, collected directly from the stercoral pocket of spiders, onto Whatman 542 filter paper.

The rate of Na and K excretion in the urine and faeces was measured by eluting the ions directly from the filter paper. The urine spots, plus the small amount of faecal material present, were cut out and dissolved in a small volume of nitric acid (Analar grade, BDH Chemicals Ltd.), then diluted with double glass distilled water and the Na and K content measured.

### I.2.2 Procedure for Sampling Haemolymph and Stercoral Fluid

Haemolymph and stercoral fluid were collected in small silicone-coated (Repelcote, 2% dimethyldichlorosilane in carbon tetrachloride) pyrex pipettes. A small amount of liquid paraffin was drawn into each pipette to prevent evaporation from the fluid during collection.

The spiders were lightly anaesthetised with CO<sub>2</sub> and restrained on their dorsal surface. A ligature was loosely tied around the metatarsal segment of either the third or fourth walking leg, the arthrodistal cuticle at the joint of the metatarsal and tarsal segments pierced with the glass pipette tip and haemolymph collected directly from the leg. Upon completion of sampling the ligature was drawn tight and the small wound in the joint covered with molten dental wax to prevent further bleeding.

Stercoral fluid was collected by inserting the tip of a pipette through the anal tubercle and up into the stercoral pocket. The pipette tip was flamed to avoid damaging the soft cuticle lining the rectal tube running from the anal tubercle to the stercoral pocket.

### I.2.3 Injection and Measurement of Isotopes

Approximately 0.074 MBq gm<sup>-1</sup> of carrier free <sup>22</sup>Na (New England Nuclear) in saline (NaCl, 200 mM l<sup>-1</sup>; KCl, 4.8 mM l<sup>-1</sup>; CaCl<sub>2</sub>, 4.5 mM l<sup>-1</sup>; MgCl<sub>2</sub>, 2.5 mM l<sup>-1</sup>; NaHCO<sub>3</sub>, 3.0 mM l<sup>-1</sup>; pH = 7.3) were injected into the spiders. Depending upon the size of the spider, between 15 and 25 µl of saline were injected.

The spider was lightly anaesthetised with CO<sub>2</sub> and restrained on its dorsal surface. To prevent bleeding during and after injection, the following procedure was adopted. A ligature was tied around the

tibial segment of the third walking leg and the needle of a 50  $\mu$ l Hamilton micro syringe inserted into the tibial/metatarsal joint and tied into the tibial segment with a second ligature. The first ligature was then released and the saline injected over a period of three to five minutes. After a further five minutes, the first ligature was retightened, the second one removed and the needle withdrawn. The tibial/metatarsal joint was then covered with dental wax.

The  $^{22}\text{Na}$  activities of the filter paper, dried cotton dental buds, which were soaked with tapwater during the experiments to provide drinking water, and the food debris were measured in an Ortec gamma well counter. The filter paper was subdivided into small pieces then placed in 7 ml plastic tubes for counting. The cotton dental buds were dried in air for 96 hours, and the food debris dried at 100°C for 48 hours before counting.

#### I.2.4 Determination of Cation Content of Tissues

Spiders were weighed then killed with ether. Tissue samples were dissected free and placed in pre-weighed 5 or 15 ml silica glass crucibles. The tissue and crucibles were then weighed, dried for 48 hours at 100°C, weighed again, then dry ashed in a muffle furnace at 600°C for 12 hours. The small amount of ash remaining was dissolved in a minimum volume of 50% nitric acid, diluted with double glass distilled water and the sodium and potassium concentrations measured.

#### I.2.5 Chemical Determination

Samples of haemolymph and stercoral fluid were stored under liquid paraffin in clean plastic petri dishes for brief periods between sampling and measurement. Osmotic pressure measurements of these fluids were made according to the method of Ramsay and Brown (1955) with the following modifications. Cooling was provided by a deep freeze probe placed directly in the bath and the thermometer was positioned within 1 cm of the side of the sample tube. Osmotic pressure measurements were made on drops of fluid interspersed with liquid paraffin in 1  $\mu$ l silicone-coated pipettes instead of the type of capillary described by Ramsay and Brown (1955).



The chloride concentrations of haemolymph and stercoral fluid were determined by potentiometric titration according to the second method devised by Ramsay *et al.* (1955).

Sodium and potassium concentrations of the haemolymph and stercoral fluid were determined with an atomic absorption spectrophotometer (Varian 1200). Aliquots of the fluids were collected in either 1  $\mu$ l or 5  $\mu$ l silicone-coated pyrex glass microcaps (Drummond Ltd.), diluted with appropriate volumes of double glass distilled water and measured against NaCl and KCl standards made up in distilled water.

The Na and K content of the excreta and ashed tissue samples were also determined with the spectrophotometer. Cations from ashed tissue samples were measured against NaCl and KCl standards made up in distilled water, and the cations eluted from the excreta against standards made up in 1%  $\text{HNO}_3$ .

#### I.2.6 Tissue Preparation for Optical and Transmission Electron Microscopy

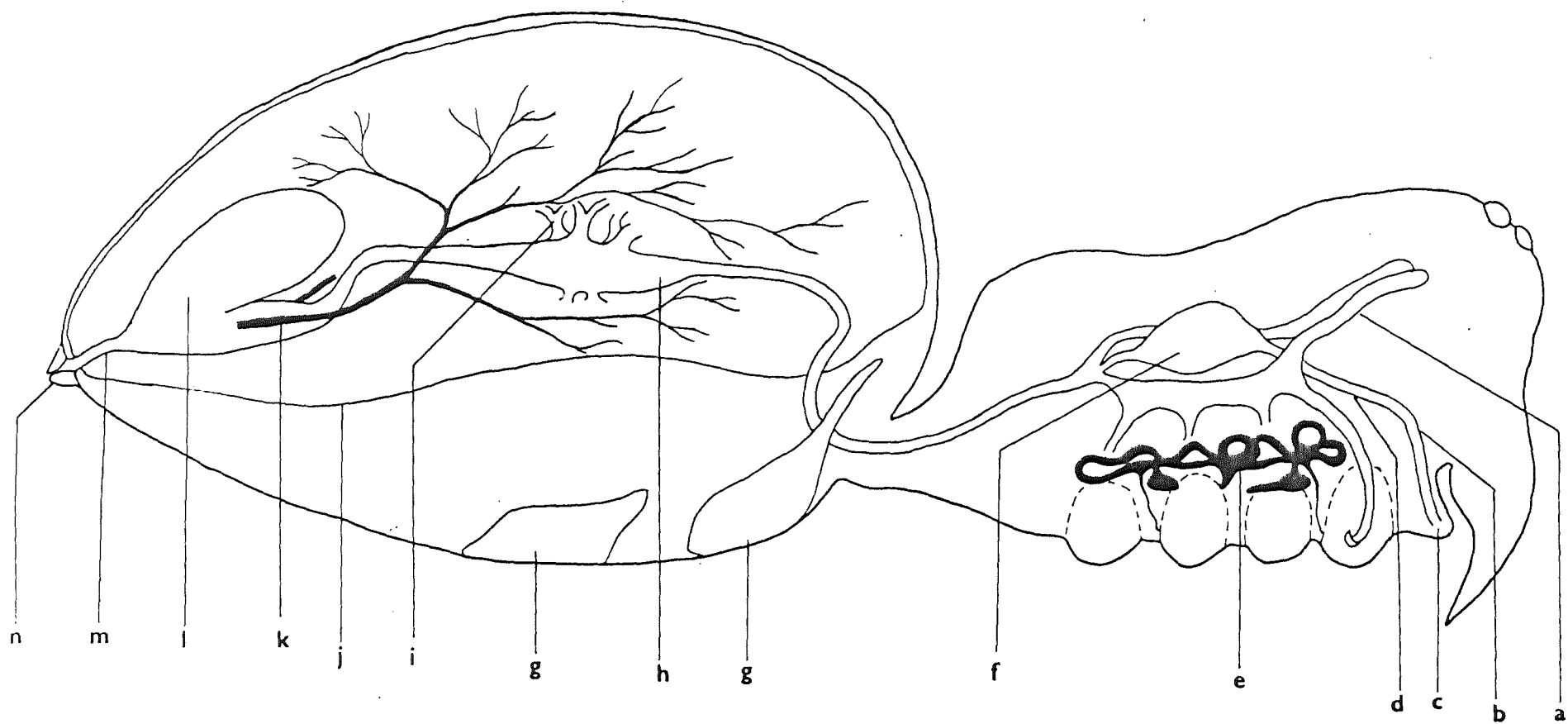
##### I.2.6.a Optical microscopy

The abdomens of freshly killed spiders were transferred to alcoholic Bouins and the abdominal cuticle removed. The tissue was then transferred to fresh fixative for 48 hours, washed in 70% ethanol saturated with  $\text{LiCO}_3$  and then stored in 70% ethanol. Before embedding, the tissue was dehydrated in an alcohol series and cleared overnight in cedarwood oil. The tissue was then vacuum embedded in paraffin wax and sectioned at 8-10  $\mu$ m on a Beck microtome. Sections were mounted on glass slides and stained with either Ehrlich haemotoxylin or Mallory triple stain.

##### I.2.6.b Transmission electron microscopy

For electron microscopy, tissue was fixed for six hours in a cold ( $4^\circ\text{C}$ ) solution containing 2.5% formaldehyde, 2.5% glutaraldehyde, 0.1 M cacodylate (pH 7.4) and 0.1 M sucrose. After washing in buffered sucrose, tissue was post-osmicated in 2%  $\text{OsO}_4$  in the same vehicle and embedded in Spurr's resin (TAAB Laboratories). Ultrathin sections were cut on a LKB Bromma 8800 Ultratome III, double stained using uranyl

Figure I.1      The relative positions and arrangement of the components of the alimentary and excretory systems in the spider *P. antipodiana*.    a = foregut diverticula; b = pharynx; c = mouth; d = oesophagus; e = coxal gland; f = pumping stomach; g = booklungs; h = central midgut tube; i = midgut diverticula; j = outer limit of tissue mass consisting of midgut diverticula and storage material; k = Malpighian tubules; l = stercoral pocket; m = rectal tube; n = anal tubercle.



acetate and lead citrate and examined using a Jeolco T.E.M. 100B electron microscope.

### I.3 RESULTS

#### I.3.1 The Anatomy of the Alimentary Canal and Histology and Ultra-structure of the Malpighian Tubules and Stercoral Pocket

The relative position in the body of the various components of the alimentary canal and excretory apparatus of *P. antipodiana* is shown in Figure I.1. The arrangement of the gut seen in *P. antipodiana* is characteristic of spiders in general. It consists of three sections, the ectodermally derived foregut and hindgut, and the endodermally derived midgut. The foregut and hindgut are lined with chitinous layers continuous with the cuticle of the body surface, while the midgut is lined with cells which form the digestive epithelium.

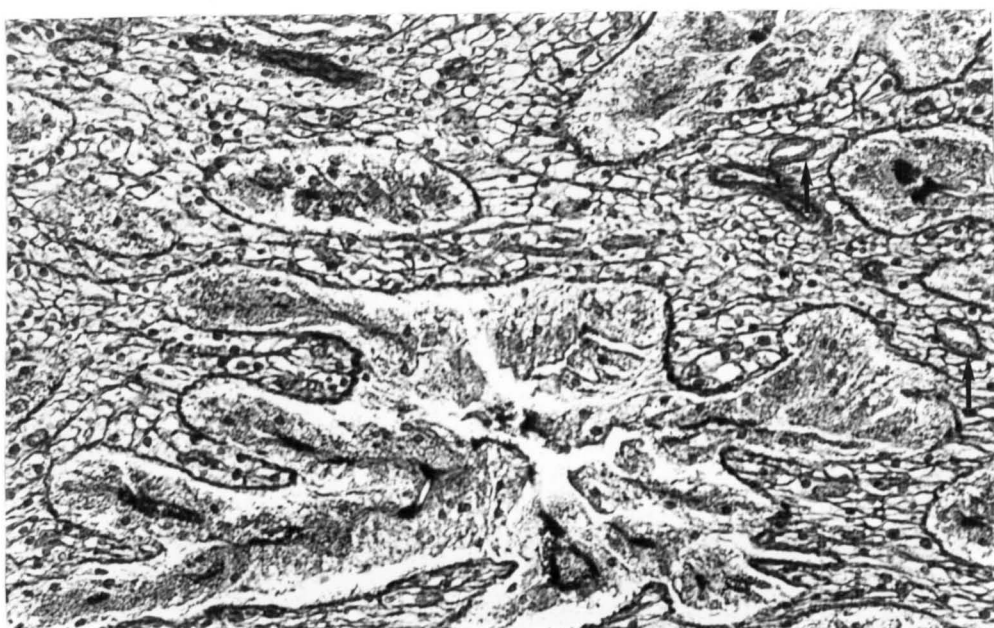
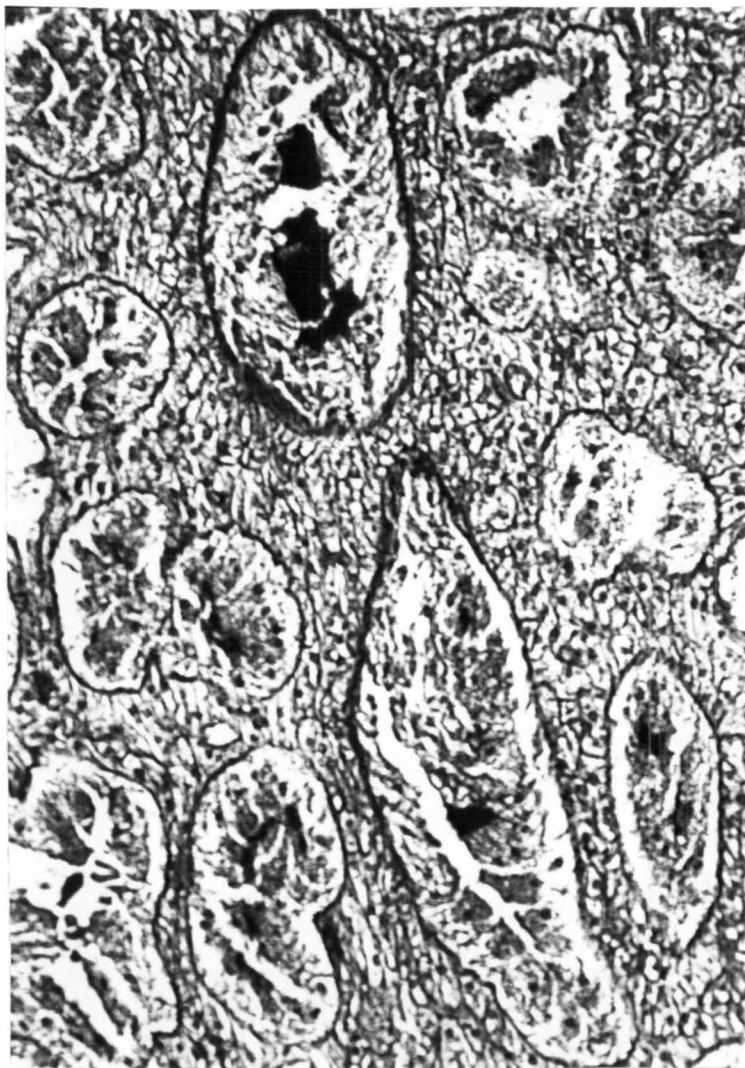
The mouth is a small opening formed at the junction of the rostrum, labium and maxillary blades (Legendre, 1978). It is suited for the ingestion of fluid and fine particles only, and is surrounded by numerous hairs, extending from the maxillary blades and labium, which function as sieving devices restricting the entry of large food particles (Comstock, 1940; Legendre, 1978).

The foregut consists of the pharynx, oesophagus and sucking stomach. The pharynx passes vertically from the mouth to the oesophagus which then passes back horizontally between the dorsal and ventral nerve masses to the sucking stomach. A series of powerful muscles attached to the pharynx and the pumping stomach expand and constrict these structures, generating the suction which is employed in the ingestion of the liquified food (Legendre, 1978).

The midgut arises directly from the pumping stomach. The characteristic feature of the midgut of *P. antipodiana*, and spiders in general, is the presence of extensive diverticula which are divided into two portions, those restricted to the cephalothorax and those restricted to the abdomen. The arrangement of the cephalothoracic diverticula of *P. antipodiana* conforms to the classical pattern as described by Millot (1931). Five pairs of tube-like diverticula are present, four of which

Figure I.2      Light micrograph of a transverse section through the abdominal tissue mass showing an individual diverticulum branching from the central midgut tube.  
(Magnification, 860x )

Figure I.3      Light micrograph of a transverse section through the abdominal tissue mass showing the extensive branching of an individual diverticulum.    Arrow indicates a Malpighian tubule.    (Magnification, 860x )



branch laterally and extend into the coxal segments of the walking legs, and one of which extends anteriorly towards the chelicerae.

Within the abdominal diverticula a central tube is present from which the individual diverticula branch. Two pairs of diverticula branch from the dorsal surface of the central tube while ventrally two diverticula branch at individual points (Figure I.2). The abdominal diverticula are not simple tube-like structures as seen in the cephalothorax but, having branched from the central midgut tube, they in turn branch extensively (Figure I.3) forming an extensive network of diverticula which fill the space defined by the heart dorsally and the reproductive organs and spinnerets ventrally. The spaces between the individual diverticula are filled with fat and storage tissue (Figure I.3), forming a discrete tissue mass.

After giving rise to the diverticula the central midgut tube is constricted and turns ventrally before entering the stercoral pocket. At the point of constriction the faecal material produced by the midgut diverticula is invested with an ensheathing membrane. Van der Bourght (1966) described similar membranes in the spiders *Linyphia triangularis* and *Araneus diadematus* and labelled them peritrophic membranes. However, the membranes observed in the gut of spiders are not homologous with the peritrophic membranes of the insects which are secreted, either throughout the midgut (e.g., phasmids, acridiids and Ephemeroptera), or by a group of cells at the anterior limit of the midgut (larval and adult Diptera), and function to protect the delicate epithelium of the midgut from abrasion by the ingested food material (Wigglesworth, 1972).

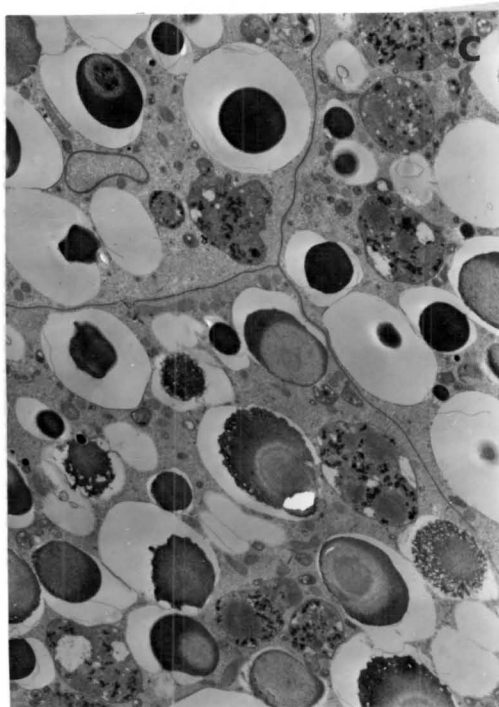
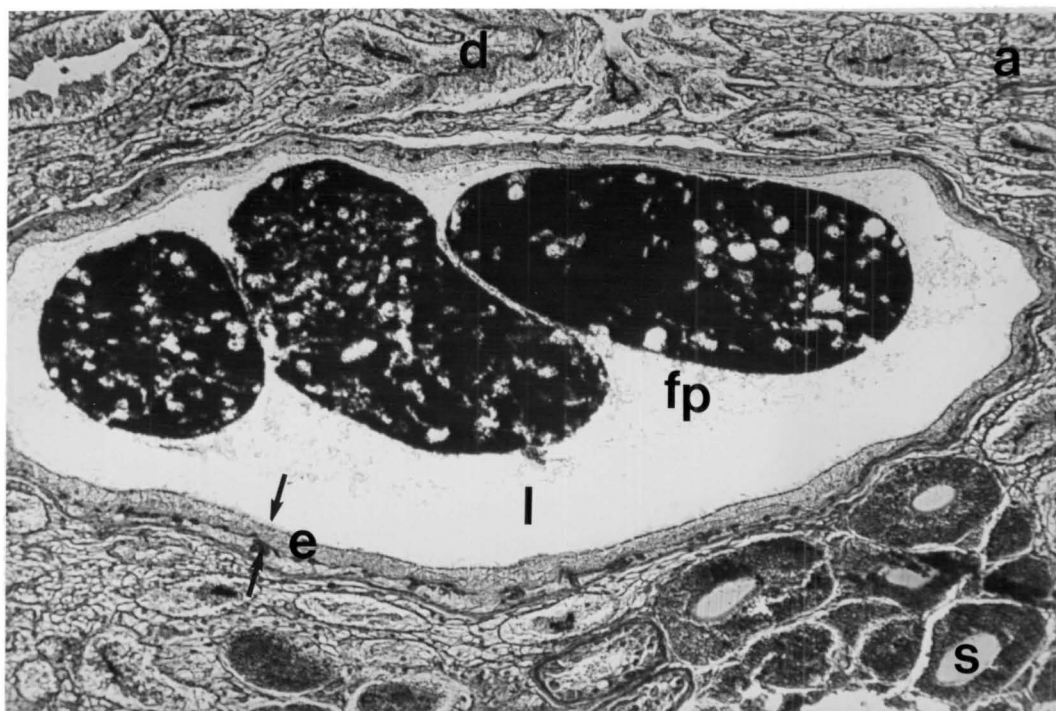
The hindgut consists of the stercoral pocket and the rectal tube. The stercoral pocket is a simple extensible sac or diverticulum in which faecal material and excretory fluid are stored before excretion (Figure I.4). There is some dispute as to whether the stercoral pocket is of ectodermal or endodermal origin. Comstock (1940) describes the stercoral pocket as a portion of the hindgut derived from the proctodaeum and lined with cuticle. Millot (1926, 1949), however, does not mention the presence of a cuticular lining in the stercoral pocket of spiders and describes it as a simple extensible diverticulum which is histologically very similar to the Malpighian tubules. In *P. antipodiana* there was no evidence of a cuticular lining, and the apical surface of the stercoral pocket was covered with loosely packed microvilli (Figure I.4).

Figure I.4a      Light micrograph of a transverse section through the stercoral pocket.    (Magnification, 640x)  
f = faecal pellet; l = lumen of the stercoral pocket;  
d = diverticula and storage material; s = silk glands;  
e = stercoral pocket epithelium.

Figure I.4b      Electron micrograph of the apical surface of the stercoral pocket showing the loosely packed micro-villi and the absence of a cuticle lining.    (Magnification, 6227x)

Figure I.4c      Electron micrograph of a transverse section through the mid region of the stercoral pocket epithelium showing the numerous concretions which are present.  
(Magnification, 4,930 x)





Numerous concretions were evident in the epithelium of the stercoral pocket (Figure I.4), although similar structures were not evident in the lumen. Similar concretions were noted in the Malpighian tubules of *P. antipodiana*, and have been reported in protozoa (Andre and Fremient, 1967) and the Malpighian tubules (Wall *et al.*, 1975) and intestinal cells (Gouranton, 1968) of insects.

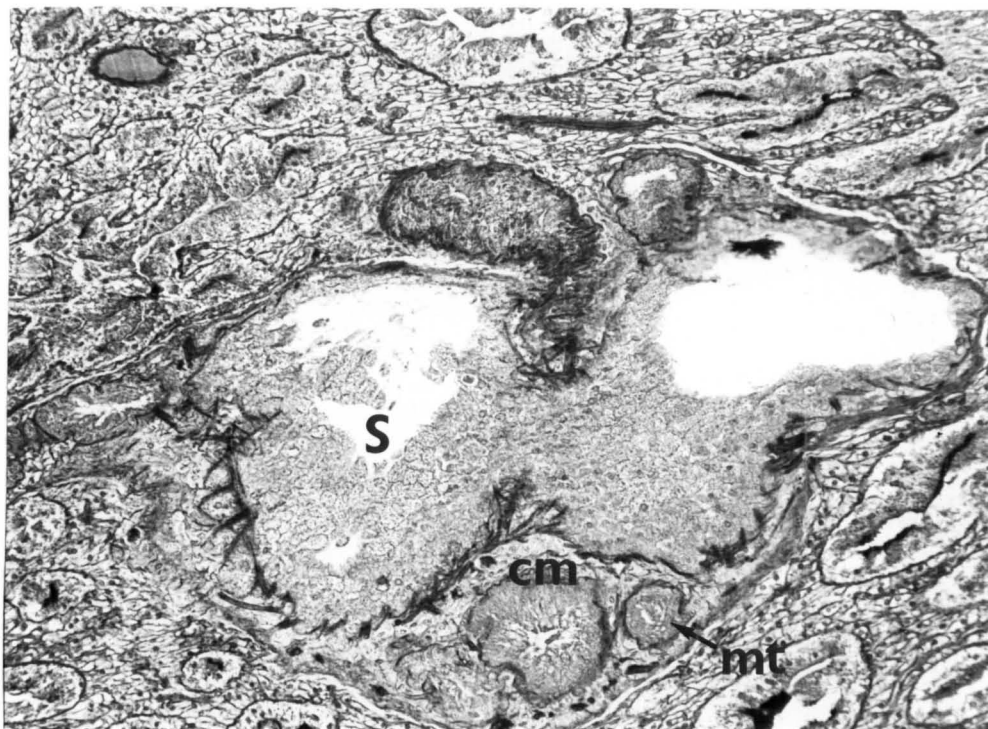
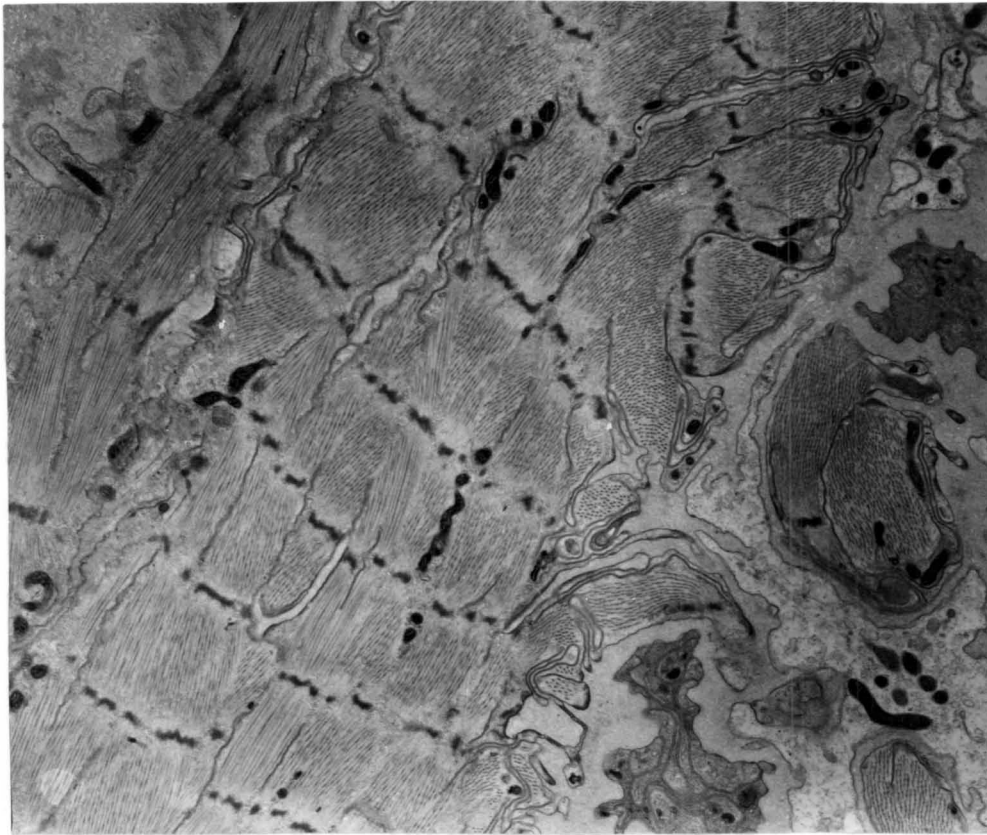
Unlike the central midgut tube the stercoral pocket is surrounded by a thin sheath of muscle. A feature of this muscle is that within any given fibre the filaments are found orientated at widely divergent angles (Figure I.5). A similar arrangement is seen in the flight control and heart muscle of the wasp, *Vespa* (Elder, 1975), and has also been noted in vertebrate smooth muscle (Devine and Somylo, 1971). The contents of the stercoral pocket pass to the exterior via the short cuticle lined rectal tube.

The Malpighian tubules, of which there are a pair, branch from the midgut (Figure I.6), and thus, unlike insect Malpighian tubules, are endodermal in origin (Clarke, 1979). The pair of tubules extend forward parallel to the central midgut tube for a short distance before branching many times in a dichotomous fashion and producing a network of fine tubules which is restricted to, and ramifies throughout, the tissue mass consisting of the abdominal diverticula and storage material. Within the tissue mass the Malpighian tubules are surrounded by fat cells and do not appear to have a close association with the diverticula of the gut (Figure I.3).

Seitz (1975) described the ultrastructure of the Malpighian tubules of the spider *Cupiennius salei*, and subdivided the tubules into three segments, initial, main and terminal. All tissue from the Malpighian tubules of *P. antipodiana*, fixed and sectioned for electron microscopy, was taken from between the second and terminal dichotomous branch, a region which corresponds to the main segment described by Seitz for *C. salei*. The characteristic feature of the tubules in this region was numerous vacuoles distributed throughout the cytoplasm, and containing mineralised concretions similar to those seen in the stercoral pocket (Figure I.7). Extensive rough endoplasmic reticulum and accumulations of ribosomes were also evident in the cytoplasm. The apical surface of the tubule cells was extended by loosely packed plate-like microvilli. The mitochondria, distributed throughout the

Figure I.5      Electron micrograph showing a transverse section through the muscle blocks in the basal region of the stercoral pocket.    (Magnification, 6,900 x)

Figure I.6      Light micrograph showing a transverse section through the abdominal tissue mass close to the junction of the stercoral pocket and the midgut.    (Magnification, 125x )  
cm = central midgut tube; mt = Malpighian tubule;  
s = stercoral pocket.



cytoplasm, were not associated with these structures and there was no evidence of the mitochondria extending into the plates. Unlike insect Malpighian tubules there were no infoldings present in the basal region of the cells, an observation which is, perhaps, not unexpected in view of the close association of the Malpighian tubules with the surrounding fat cells. It is worth noting that, unlike the Malpighian tubules of insects which lie free in the haemolymph, those of the spiders are restricted to the abdominal tissue mass and closely associated with surrounding fat cells.

A second potential excretory system, the coxal glands, is located in the cephalothorax of spiders (Buxton, 1913; Millot, 1949). The position of the coxal glands in the cephalothorax of *P. antipodiana* is shown in Figure I.1 and its structure is described in Section II.

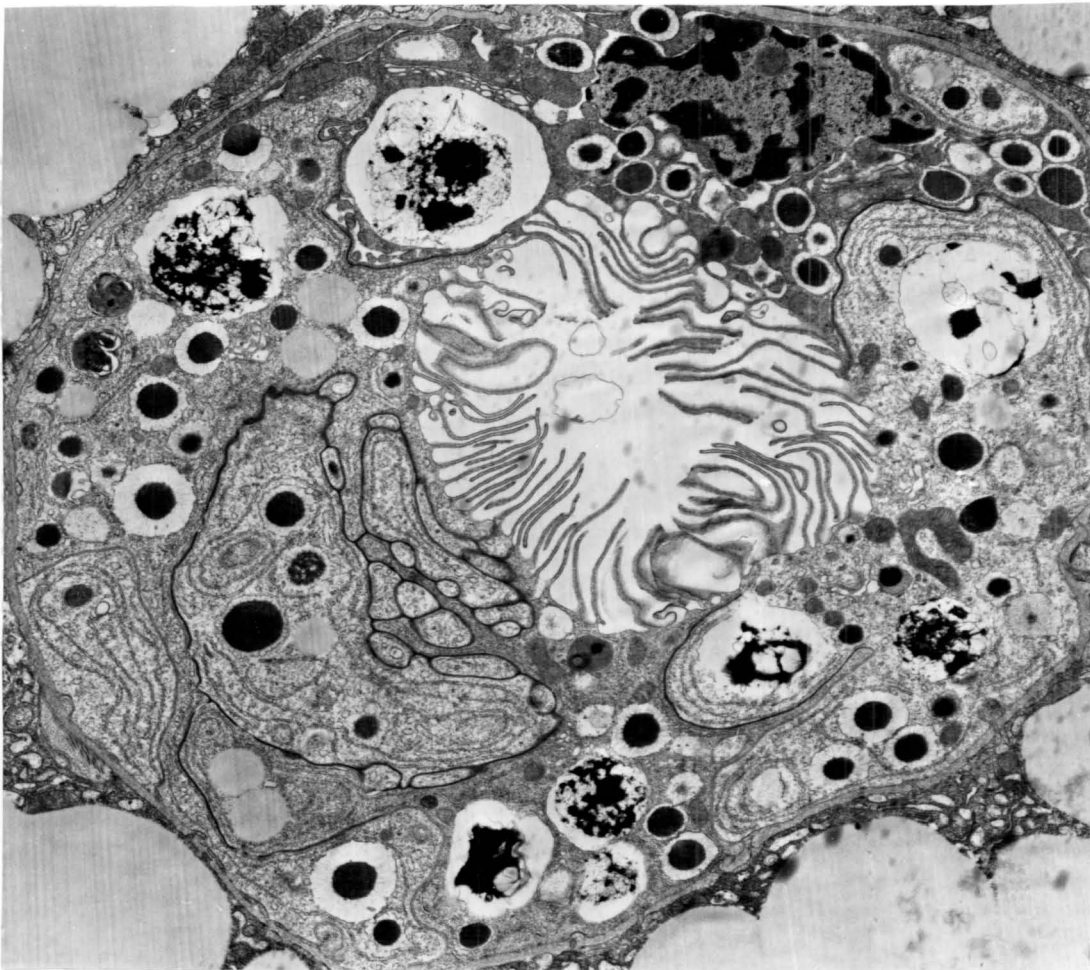
### I.3.2 Definitions

There is no established terminology to describe the fluids produced by the excretory system of spiders, and while it is tempting to adopt the terminology employed with the insects, this would not necessarily be precise. For example, labelling the fluid collected from the stercoral pocket, rectal fluid, would be misleading as, although the position of the stercoral pocket is reminiscent of the rectum of insects, it is not a structurally homologous organ as it does not have a cuticular lining (Millot, 1949; this study). Furthermore, there is no evidence that its function is physiologically the same as the rectum. Therefore, to avoid ambiguity the following terms have been employed when referring to the various excretory fluids of *P. antipodiana*:

- (i) Stercoral fluid - fluid collected directly from the stercoral pocket.
- (ii) Urine - fluid voided via the anus by the spider.
- (iii) Excreta - urine and solid material (mainly faeces in the form of pellets) voided via the anus by the spider.

It was also necessary to define the midgut diverticula and their associated Malpighian tubules and stercoral pocket as a single excretory system, the anal system. This need arose from the close association of the Malpighian tubules and stercoral pocket with the surrounding

Figure I.7      Electron micrograph of a transverse section through a Malpighian tubule.    (Magnification, 3,900 x)



diverticula and fat cells. This association prevented the complete or partial isolation of the Malpighian tubules into ringer solutions and a subsequent *in vitro* investigation of their operation. Furthermore, as the Malpighian tubules and central midgut tube were completely encased within the diverticula and storage material, it was not possible to obtain reliable fluid samples from the tubules or gut tube. Therefore it was only possible to state that the stercoral fluid and urine was derived from the anal system, i.e., the Malpighian tubules, stercoral pocket and midgut diverticula.

### I.3.3 Changes Produced by Feeding of *P. antipodiana*

To investigate the role of the anal system in the water balance and ionic regulation of normal hydrated *P. antipodiana*, changes with feeding in the body weight, ion content and rates of anal urine production and cation excretion were measured. Spiders were starved, with water, for three weeks, then divided into two groups of similar mean body weight (controls,  $0.8608 \pm 0.05$  gm; experimental,  $0.8536 \pm 0.04$  gm;  $X \pm S.E.M.$ ,  $n = 8$  for each group). One group was provided with a constant supply of water and live cockroach nymphs for five days (experimental animals), while the second group was starved with water for a further five days (control animals). During the five day experimental period the spiders were weighed, and the rate of anal urine production and cation excretion determined daily. At the completion of the period, haemolymph samples and the contents of the stercoral pocket were collected from all spiders. The spiders were then killed and the abdominal diverticula, containing the Malpighian tubules and the emptied stercoral pocket, were dissected free from the rest of the body. The water and cation content of this tissue mass was determined separately from the rest of the body. As the two groups were of similar mean weight, and as they were treated in the same manner prior to feeding, differences between the two groups after feeding should represent the effects of feeding.

#### I.3.3.a Weight changes with feeding

In Table I.1 the mean live weight, the mean weight of dry material and the mean weight of water of the two groups after the period of feeding or starvation are shown.

Feeding *P. antipodiana* showed a reasonably constant daily increase



Table I.1 The mean body weight, wet weight and dry weight of two groups of spiders selected so that the initial mean weights of both groups were the same after three weeks of starvation. One group was subsequently fed cockroaches for five days while the second group was starved a further five days. ( $\bar{X} \pm \text{S.E.M.}$ )

	Starved spiders (n = 8)	Fed spiders (n = 8)	Percentage increase	Percentage of increase due to	
				Abdominal diverticula*	Rest of body**
Live weight (gm)	0.8608 $\pm$ 0.0510	1.2578 $\pm$ 0.059	46	84	15.8
Weight of water (gm)	0.6309 $\pm$ 0.094	0.8733 $\pm$ 0.1230	37	78	22
Weight of dry material (gm)	0.2189 $\pm$ 0.020	0.3810 $\pm$ 0.020	74	93	6.5

\* Abdominal diverticula includes Malpighian tubules and stercoral pocket.

\*\* Rest of body includes cephalothorax, abdominal cuticle, ovaries, heart and spinnerets.

in body weight and after five days their weight had increased 46% compared to control starved spiders. Predictably the increase in body weight was accounted for by increases in the water content and dry material of the fed spiders, however, the dry material content increased by 74%, almost double the 37% increase in water content.

An obvious effect of feeding on *P. antipodiana* was a large increase in the size of the abdomen relative to the cephalothorax. In *P. antipodiana*, as with most spiders, the bulk of the abdominal contents consists of diverticula, and in fed spiders increases in the weight of the abdominal diverticula were responsible for most of the observed changes in the wet and dry weights. Of the total weight increase of the fed animals, 84% was restricted to the abdominal diverticula. Similarly, 78% of the total increase in water content, and 93% of the total increase in dry material were due to increases in the abdominal diverticula (Table I.1). Consequently, in fed spiders the abdominal diverticula constituted 40% of the total body weight, whereas in the starved animals it only represented 19% of the body weight.

#### I.3.3.b Changes in the relative water content with feeding

The percentage increase in the dry material with feeding was almost twice that of water (Table I.1 and previous section) resulting in a reduction in the relative water of the whole spiders from 74.8% to 69.5%. Most of these changes appeared to take place in the abdominal diverticula (Table I.2). In fed spiders the relative water content of the diverticula decreased from 66.1% to 58.1%, whereas there was actually a small increase in the relative water content of the rest of the body.

#### I.3.3.c Changes in the amount and concentration of Na and K in the body of *P. antipodiana* with feeding

The mean Na and K contents of starved and fed groups of spiders are shown in Table I.3. With feeding there were increases of about the same magnitude in the amount of Na and K present in the whole body of the spiders. Potassium increased from 48.1 to 90.4  $\mu\text{M}$ , while Na increased from 60.8 to 98.4  $\mu\text{M}$ . However, the relative K content increased by 90% compared to only a 60% increase in the relative Na content, so that the

Table I.2 Percentage water content of whole spiders, the abdominal diverticula plus Malpighian tubules and stercoral pocket and the rest of the body. Both groups of spiders were treated in a similar manner and were a similar mean weight prior to the experiment. Fed spiders provided with food and water for five days, starved spiders provided with water only. ( $\bar{X} \pm \text{S.E.M.}$ ,  $n = 8$ )

	Percentage Water Content		
	Whole spider	Abdominal diverticulat	Rest of body††
Starved spiders	$74.8 \pm 0.74$	$66.1 \pm 2.1$	$75.2 \pm 0.5$
Fed spiders	$69.5 \pm 1.0^{***}$	$58.4 \pm 1.3^{**}$	$77.0 \pm 0.9^{\text{N.S.}}$

Significance level of differences when means of fed spiders tested against starved spiders using unpaired Student  $t$  test. N.S. = not significant;  $** = 0.01 > p > 0.001$ ;  $*** = 0.001 > p$ .

† Abdominal diverticula includes Malpighian tubules and stercoral pocket epithelium.

†† Rest of body includes cephalothorax, abdominal cuticle, spinnerets, heart and ovaries.

Na/K ratio of the whole body decreased with feeding from 1.32 to 1.08. These trends were even more marked when the abdominal diverticula alone were examined. With feeding, the K content of the abdominal diverticula increased markedly from 12.8 to 40.3  $\mu\text{moles}$  but the Na content only increased slightly from 8.6 to 15.2  $\mu\text{moles}$ . Consequently, the Na/K ratio of the abdominal diverticula of fed spiders (0.39) was only slightly more than half that of starved spiders (0.69). Conversely, in the rest of the body the increase in Na content with feeding (55.2 to 83.3  $\mu\text{M}$ ) exceeded the increase in K content (35.2 to 51.1  $\mu\text{M}$ ) resulting in an increased Na/K ratio of these tissues.

Also in Table I.3 are listed the Na and K concentrations, expressed as mMoles (kg wet weight)<sup>-1</sup>, in the whole spider, the abdominal diverticula which includes the Malpighian tubules and stercoral pocket, and the rest of the spider's body.

In fed spiders there was a marked increase in the mean K concentration of the whole body from 55.0 to 72.6 mM kg<sup>-1</sup>, but there was only a slight increase in the Na concentration from 71.0 to 78.6 mM kg<sup>-1</sup>.

Within the cephalothorax, abdominal cuticle, ovaries, spinnerets and heart (the rest of the body) both the Na and K concentrations increased in fed spiders. But, despite observations that the amounts of Na and K in the abdominal diverticula increased substantially with feeding, the K concentration of the diverticula showed little change in fed spiders, while the Na concentration actually decreased by almost half. These observations are, however, consistent with observed changes in the water content of the tissues, as the wet weight of the abdominal diverticula increased markedly while the rest of the body showed little change.

#### I.3.3.d The effects of feeding on the composition of the haemolymph and stercoral fluid of hydrated spiders

The major osmolar effectors in the haemolymph of *P. antipodiana* were Na and Cl, which in the starved spider accounted for 48% and 35% respectively of the total haemolymph osmotic pressure (Table I.4). Potassium was present at very low concentrations and, although not shown in Table I.4, so were Ca<sup>2+</sup> and Mg<sup>2+</sup> (Ca<sup>2+</sup> = 5.1 mM l<sup>-1</sup>; Mg<sup>2+</sup> = 3.6 mM l<sup>-1</sup>;  $\bar{X}$ , n = 3).

Table I.3 The  $\mu$ Moles and concentration of Na and K in the whole body, abdominal diverticula plus Malpighian tubules and stercoral pocket and the rest of the body of starved and fed spiders. Both groups of spiders were treated in a similar manner and were a similar mean weight prior to the experiment. Fed spiders provided with food and water for five days, starved spiders provided with water only. ( $X \pm S.E.M.$ ,  $n = 8$ )

	Starved spiders	Fed spiders	Abdominal Diverticula†		Rest of Body††	
			Starved	Fed	Starved	Fed
$\mu$ Moles Potassium	$48.0 \pm 5.0$	$91.4 \pm 5.5^{***}$	$12.8 \pm 1.8$	$40.3 \pm 2.0^{***}$	$35.2 \pm 3.3$	$51.1 \pm 3.9^{**}$
mMoles (kg wet tissue) $^{-1}$	$55.0 \pm 3.0$	$72.6 \pm 1.8^{***}$	$79.6 \pm 10.3$	$81.1 \pm 2.5$	$50.5 \pm 3.3$	$67.1 \pm 2.8^{**}$
$\mu$ Moles Sodium	$60.8 \pm 3.2$	$98.4 \pm 6.7^{***}$	$8.6 \pm 0.91$	$15.2 \pm 0.92^{***}$	$52.2 \pm 2.6$	$83.3 \pm 6.8^{***}$
mMoles (kg wet tissue) $^{-1}$	$71.0 \pm 6.5$	$78.1 \pm 3.3$	$54.8 \pm 3.7$	$31.0 \pm 2.2^{***}$	$75.2 \pm 2.7$	$109.7 \pm 6.2^{***}$
Na/K	$1.32 \pm 0.098$	$1.08 \pm 0.05^*$	$0.69 \pm 0.03$	$0.38 \pm 0.02^{***}$	$1.53 \pm 0.13$	$1.64 \pm 0.09$

Significance levels of differences between means when means of fed spiders tested against means of starved spiders using Student  $t$  test.  $*$  =  $0.05 > p > 0.01$ ,  $**$  =  $0.01 > p > 0.001$ ,  $***$  =  $0.001 > p$ .

† Abdominal diverticula includes Malpighian tubules plus stercoral pocket epithelium.

†† Rest of the body includes cephalothorax, abdominal cuticle, spinnerets, heart and ovaries.

The haemolymph K concentration of fed spiders ( $4.5 \text{ mM l}^{-1}$ ) showed a slight decrease when compared to that of starved spiders ( $5.08 \text{ mM l}^{-1}$ ). However, the osmotic pressure of the haemolymph increased with feeding and increases in the concentrations of the two major inorganic ions present in the haemolymph, Na ( $196 - 225 \text{ mM l}^{-1}$ ) and Cl ( $155 - 225 \text{ mM l}^{-1}$ ) appeared to be responsible for this increase.

The stercoral fluid in both starved and fed spiders was slightly, but significantly, hypo-osmotic to the haemolymph (paired Student  $t$  test comparing stercoral fluid and haemolymph values for each individual within the two groups separately; for both groups  $0.001 > p$ ). It is interesting, however, that a significant proportion of the stercoral fluid osmotic pressure is contributed by something other than the major haemolymph inorganic ions as Na, K and Cl contribute only 40% of the stercoral fluid osmotic pressure in starved spiders and 47% in fed spiders.

Feeding had a number of effects on the composition of the stercoral fluid. The osmotic pressure increased, although as mentioned above it remained marginally hypo-osmotic to the haemolymph; the K concentration almost doubled ( $64.3$  to  $125.4 \text{ mM l}^{-1}$ ) and there was an increase, albeit a small one, in the Cl concentration from  $56.5$  to  $78.3 \text{ mM l}^{-1}$ . However, rather unexpectedly, there was a decrease in the stercoral fluid Na concentration from  $50.5$  to  $34.6 \text{ mM l}^{-1}$ . This, plus the observed increase in K concentration, resulted in a marked reduction in the Na/K ratio of the stercoral fluid of fed spiders ( $0.28 \pm 0.07$ ;  $\bar{X} \pm \text{S.E.M.}$ ,  $n = 9$ ) when compared to starved spiders ( $1.04 \pm 0.28$ ;  $\bar{X} \pm \text{S.E.M.}$ ,  $n = 9$ ).

It would be expected that the relative amounts of Na and K ingested and excreted by a feeding animal would correspond, otherwise, an ionic imbalance would result. Thus, at first sight it is surprising to find that in feeding *P. antipodiana* the Na/K ratio of the stercoral fluid decreased to such an extent that it was significantly lower than the Na/K ratio of the food provided ( $0.46 \pm 0.02$ ;  $\bar{X} \pm \text{S.E.M.}$ ,  $n = 20$ ). It is of course possible that the stercoral fluid could be modified further within the stercoral pocket, either by secretion of Na or reabsorption of K, before being excreted as final urine, and thus it is, in fact, not representative of the final excretory fluid. However, the observed composition of the final excreta (urine and faeces) voided by

Table I.4 The composition of the haemolymph and stercoral fluid collected from fed or starved spiders. Fed spiders provided with food and water for five days, starved animals with water only for five days.

	Starved with Water (n = 8)		Fed with Water (n = 8)	
	Haemolymph	Stercoral Fluid	Haemolymph	Stercoral Fluid
Osmotic Pressure mOsmoles (kgH <sub>2</sub> O) <sup>-1</sup>	436 ± 5.2	427 ± 5.5	515 ± 9.9***	504 ± 9.6 <sup>†††</sup>
Na mM l <sup>-1</sup>	196.3 ± 3.9	50.5 ± 11.5	226 ± 5.3***	34.6 ± 8.1
K mM l <sup>-1</sup>	5.08 ± 0.2	64.3 ± 12.1	4.5 ± 0.26	125.4 ± 6.1 <sup>†††</sup>
Cl mM l <sup>-1</sup>	155.7 ± 4.8	56.5 ± 12.9	225 ± 2.7***	78.3 ± 8.0
Na/K	38.9 ± 2.2	1.04 ± 0.28	51.7 ± 2.9**	0.28 ± 0.07 <sup>†††</sup>

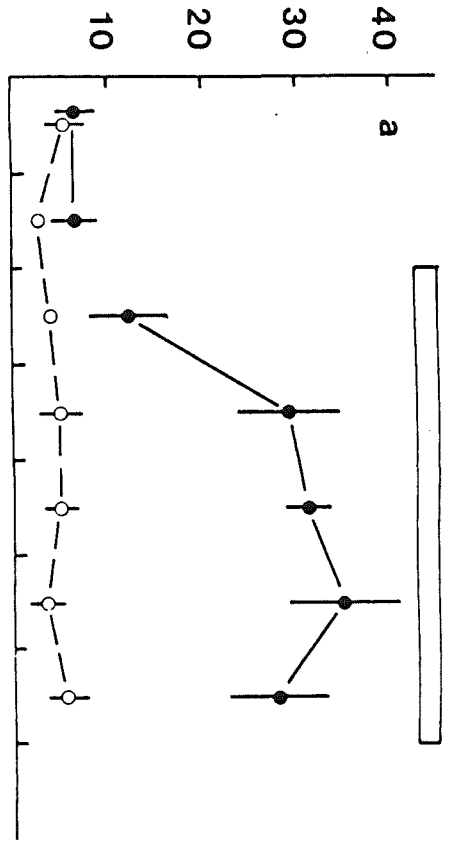
\* Comparison of means of the haemolymph of fed and starved spiders by Student t test. \* = 0.05 > p > 0.01, \*\* = 0.01 > p > 0.001, \*\*\* = 0.001 > p.

Comparison of means of stercoral fluid of fed and starved spiders by Student t test. † = 0.05 > p > 0.01, †† = 0.01 > p > 0.001, ††† = 0.001 > p.

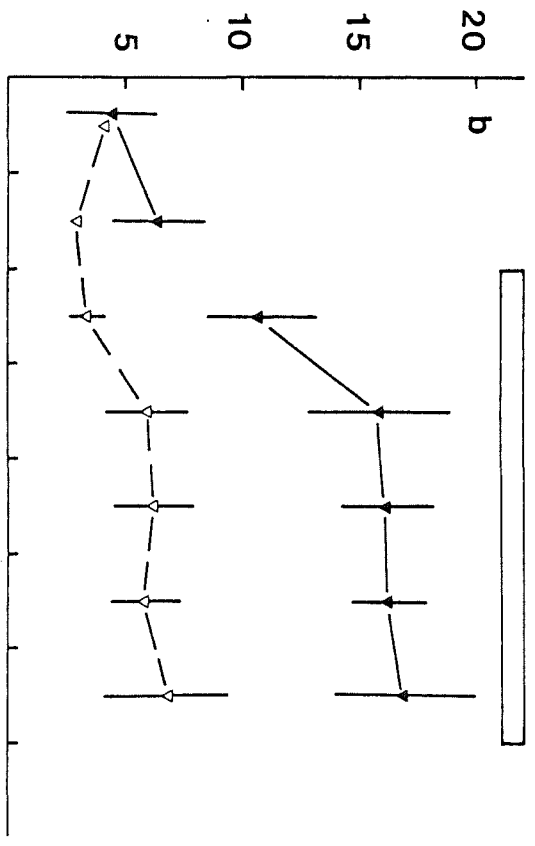
Figure 1.8      The effect of feeding on the excretion of urine by *P. antipodiana*. (a) Rate of urine excretion. (b) Volume of urine per defecation. (c) Frequency of excretion. Horizontal bar represents feeding period. Vertical lines =  $\pm 1$  S.E.M. Open symbols, control spiders provided with water only,  $n = 8$ . Closed symbols, experimental spiders provided with water plus live cockroach nymphs as food,  $n = 8$ .



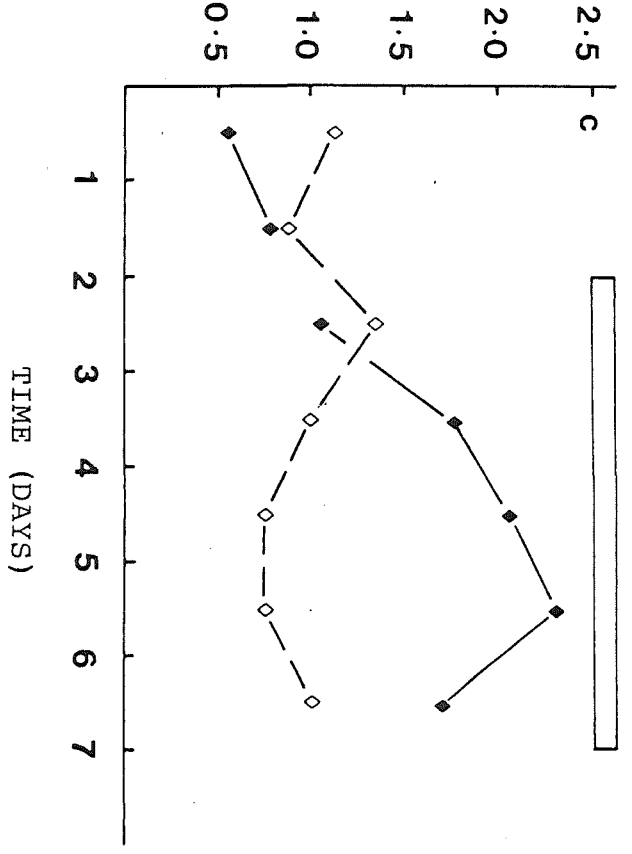
RATE OF URINE EXCRETION  
VIA THE ANUS ( $\mu\text{L DAY}^{-1}$ )



$\mu\text{L PER DEFECATION}$



FREQUENCY OF EXCRETION



feeding *P. antipodiana* does not support this proposal. Indeed, the Na/K ratio of the final urine is lower still. The significance of these observations is returned to later.

#### I.3.3.e The effect of feeding on the rate of urine production

Discrete spots of dried urine with small numbers of faecal pellets were obvious on the filter paper lining the experimental chambers of all spiders. Blockage of the anal tubercle of several spiders with dental wax confirmed that this material was voided from the anus.

Hydrated starved spiders excreted between 2.5 and 5  $\mu\text{l}$  of urine  $\text{day}^{-1}$ . The provision of food resulted in a dramatic increase in the rate of urine excretion. Within 48 hours of the onset of feeding, urine excretion had increased to 30  $\mu\text{l}$   $\text{day}^{-1}$  and daily rates equivalent to or greater than this were maintained throughout the feeding period (Figure I.8a).

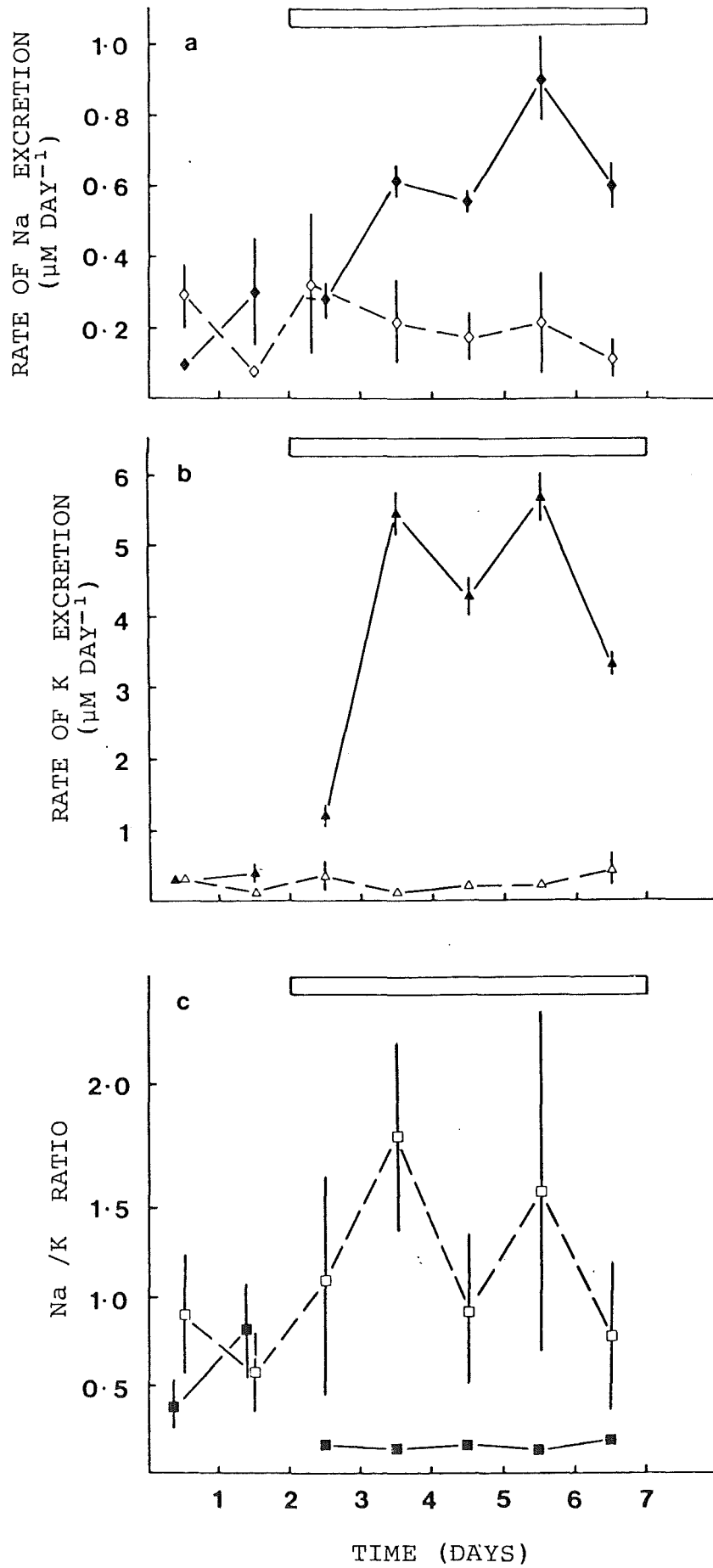
The increased rate of urine excretion of fed spiders resulted from increases in both the volume of urine excreted per defaecation (Figure I.8b) and the frequency of excretion (Figure I.8c).

#### I.3.3.f The effect of feeding on the rate of Na and K excretion

Associated with the diuresis which occurred during feeding were increases in the rates of both Na and K excretion (Figure I.9). However, the most striking effect of feeding, on the composition of the excreta, was the change in the Na/K ratio (Figure I.9). In hydrated starved control spiders the mean Na/K ratio of the excreta showed wide daily variability, ranging from 0.38 to 1.7. Furthermore, there was significant variability between individuals as indicated by the error bars in Figure I.9. However, with the onset of feeding, the Na/K ratio of the excreta fell immediately to 0.16 and was maintained at or about this level with little daily (0.13 - 0.18 range) or individual variation throughout the feeding period.

The reduced Na/K ratio of the excreta of feeding spiders resulted from the observed large increase in K excretion and a smaller increase in Na excretion. The maximum rate of K excretion of feeding spiders was 5.65  $\mu\text{M}$   $\text{l K day}^{-1}$  and a total of 19.9  $\mu\text{M}$  K were excreted by the spiders

Figure I.9      The effect of feeding on the excretion of Na and K via the anus by *P. antipodiana*.    (a) Rate of Na excretion.    (b) Rate of K excretion.    (c) Na/K ratio of the excreta. Horizontal bar represents feeding period. Vertical lines =  $\pm 1$  S.E.M. Open symbols, control spiders provided with water only, n = 8. Closed symbols, experimental spiders provided with water plus live cockroach nymphs as food, n = 8.



during the five day period, whereas, the maximum daily rate of Na excretion was  $0.9 \mu\text{M}$  and a total of only  $3.0 \mu\text{M}$  of Na was excreted.

These results were consistent with observations reported above that the stercoral fluid has a rather low Na/K ratio (Table I.4). Furthermore, the mean Na/K ratio of the excreta of fed spiders (0.159), like that of the stercoral fluid, was significantly less than the Na/K ratio of the food provided, implying that feeding spiders were not in ionic balance. In fact, these observations on the composition of the excreta suggest that feeding spiders should have been either accumulating Na or depleting themselves of K. In contrast, measurements of the cation content of feeding spiders (Section I.3.3.c, Table I.3) indicated that the K content increased to a greater extent than the Na content.

Potential explanations for these apparently contradictory results are that Na is excreted via some route other than the anus or, alternatively, K rich regions of the prey are selectively ingested.

#### I.3.4 The Ion Balance of Feeding Spiders

To test the possibility of an alternative route of Na excretion, a further series of observations was made in which the excretion and accumulation of ions by the spiders were related more precisely to the amount of ions ingested.

Spiders were provided with a meal of known Na and K content, and for each ion the total ingested was compared with the sum of the quantity voided via the anus and any change in the total ion content of the fed spiders.

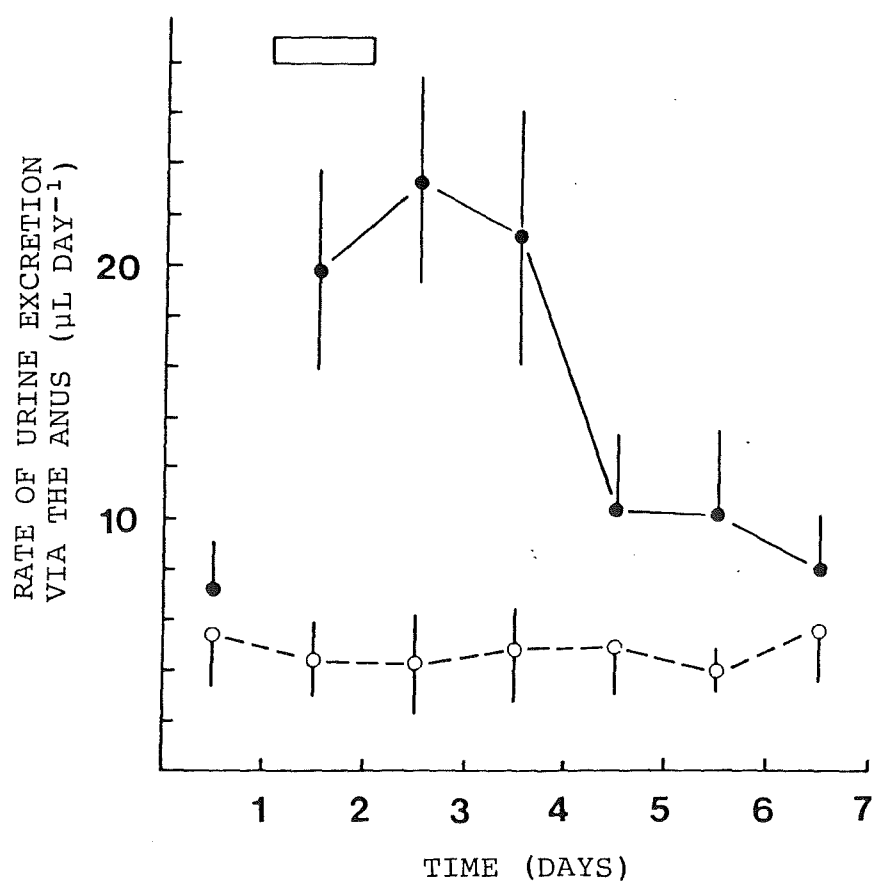
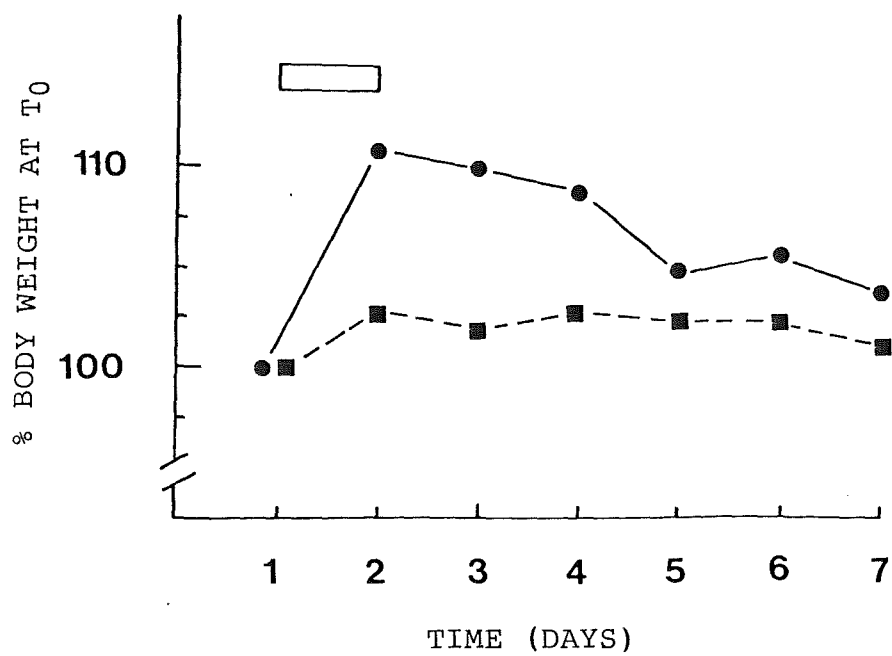
Sixteen spiders of similar weight were starved, with water, for three weeks, then divided into two groups of equal weight ( $0.7703 \pm 0.09$  and  $0.7814 \pm 0.07$ ;  $\bar{X} \pm \text{S.E.M.}$ ). One group served as a control, the second as the experimental group. The experimental spiders were starved for a further day, during which the rate of urine production and anal excretion of Na and K were determined. Each spider was then provided with three cockroach nymphs for 24 hours. The anal excretion of Na and K, and the production of urine, were measured during feeding and for five days following the completion of the meal. Water was

Figure I.10 Percentage change in the bodyweight of spiders provided with a single meal of cockroach nymphs. Horizontal bar represents feeding period.

- — — — ■ Control spiders, hydrated and starved, n = 8.
- — — — ● Experimental spiders, hydrated and fed three cockroach nymphs for 24 hours, n = 8.

Figure I.11 Effect of a single meal on the rate of urine excretion by *P. antipodiana*. Horizontal bar represents the period of feeding. Vertical bars =  $\pm 1$  S.E.M.

- — — — ○ Control spiders, starved and hydrated, n = 8.
- — — — ● Experimental spiders, hydrated and fed three cockroach nymphs for 24 hours, n = 8.



available to the spiders at all times. The control spiders were starved with water for the seven days of the experiment and their rates of urine production and anal excretion of Na and K measured daily.

The total amounts of Na and K available in the cockroach nymphs were calculated from regression curves relating the live weights of cockroach nymphs to their Na and K content. To ensure that significant quantities of Na and K were ingested with a meal, three cockroach nymphs, of known weight, were made available to each spider for a period of 24 hours. Each spider captured and fed on at least two nymphs.

Feeding by *P. antipodiana* was prolonged, ingestion of the prey requiring up to three hours depending on its size. Although the prey were thoroughly macerated during feeding, they were not completely ingested and variable amounts of debris, consisting of an amorphous grey paste, were left on completion of the meal. Thus, the net ingestion of cations with a meal was the difference between the calculated content of the whole cockroach nymphs and the measured cation content of the food debris. The net gain of Na and K by the fed spiders was estimated by comparing their Na and K content to those of the control starved spiders. Anal Na and K excretion associated with feeding was calculated as the difference between the basal rates, exhibited by the control starved spiders, and the increased rates shown by the fed spiders.

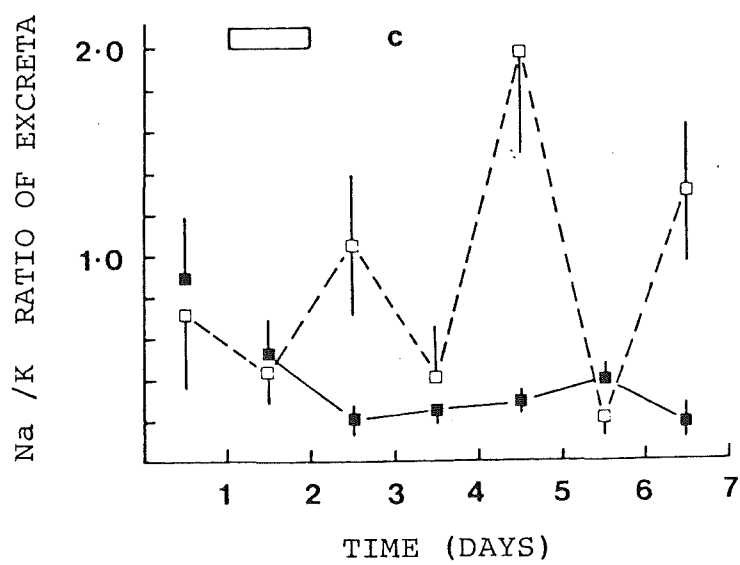
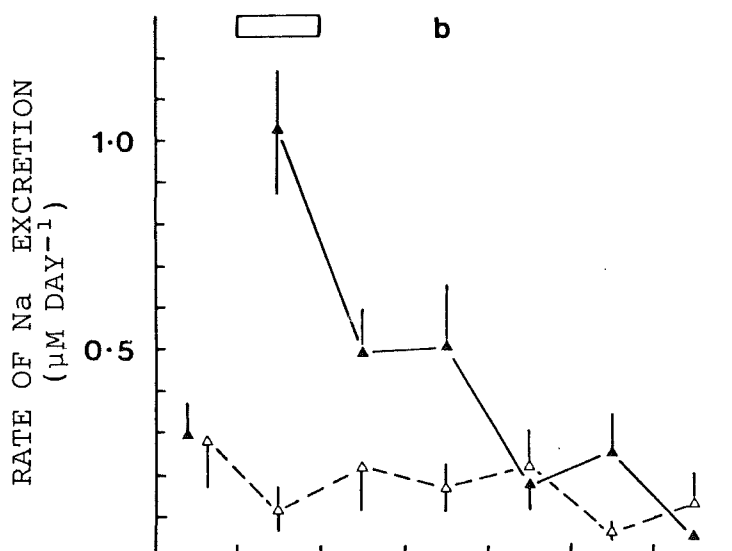
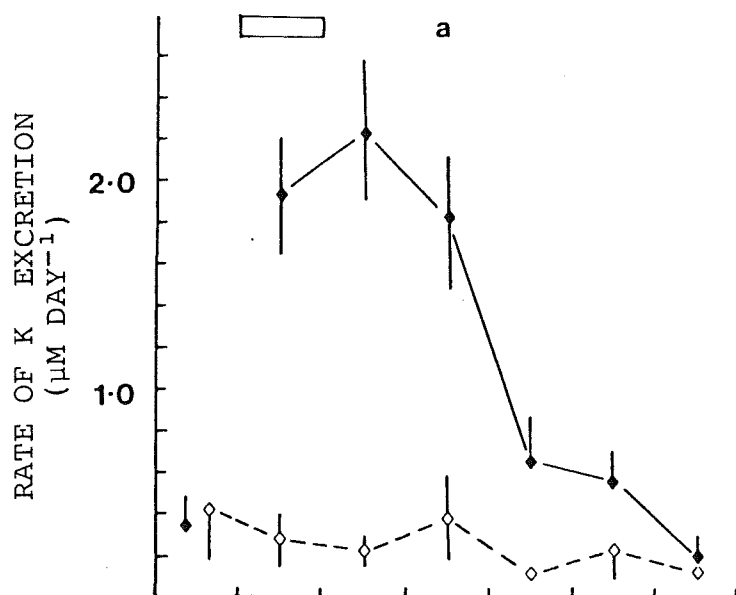
In a similar experiment the effect of feeding on the haemolymph composition of spiders was monitored. Two groups of animals were employed, one starved and hydrated, the second hydrated and fed a single meal as above. Serial samples of haemolymph were collected for both groups, samples immediately before and after the feeding period, and then two and five days after the completion of the meal.

There was an 11% increase in the body weight associated with the single meal (Figure I.10). In the two days immediately following feeding, during which anal diuresis occurred, there was only a slight reduction in body weight, but on the third day it fell to a level just above that of control spiders and was maintained at or about this level for the remainder of the experiment.

The effects of a single meal on anal urine production are shown in Figure I.11. During, and for two days following the meal, the fed



Figure I.12      Effect of a single meal on the excretion of Na and K via the anus by *P. antipodiana*.    (a) Rate of K excretion.    (b) Rate of Na excretion.    (c) The Na/K ratio of the excreta.    Horizontal bar represents feeding period.    Vertical bars =  $\pm 1$  S.E.M.    Open symbols, control spiders, hydrated and starved, n = 8.    Closed symbols, experimental spiders, hydrated and provided with three cockroach nymphs for 24 hours, n = 8.



spiders exhibited a period of anal diuresis of similar magnitude to that shown by spiders which were feeding continuously. Three days after the completion of the meal the rate of urine production decreased but remained above the control levels. Five days after the completion of the meal, the rate of urine production approached that of the control spiders.

It was noted above (Section I.3.1.d, Table I.4) that there were obvious differences in the haemolymph composition of starved and fed spiders. Changes that occurred in the haemolymph composition following a single meal were consistent with these observations (Figure I.13). On completion of the meal the haemolymph Na concentration was elevated and the K concentration reduced compared to the starved controls. During the diuretic period following feeding, the haemolymph Na concentration decreased and five days after the completion of the meal had returned to levels equivalent to that of control spiders. The K concentration, however, remained below that of the control spiders for the duration of the experiment.

Associated with the anal diuresis following feeding was a large increase in K excretion, the rate rising to a maximum of  $2.22 \mu\text{M day}^{-1}$  the day after the completion of the meal (Figure I.12). Thereafter, K excretion decreased in a similar fashion as the urine excretion, returning to control levels five days after the completion of the meal. The effect of feeding on the rate of Na excretion was not as significant. A peak in anal Na excretion ( $1.01 \mu\text{M day}^{-1}$ ) occurred during feeding, but during the day following feeding, when K excretion was greatest, Na excretion dropped markedly and had returned to control levels three days after the completion of the meal (Figure I.12).

As was noted for spiders provided with a constant source of food, feeding had a marked effect on the Na/K ratio of the excreta. The Na/K ratio of the control spiders was highly variable, ranging between 0.2 and 2.0. Following feeding, the Na/K ratio of the experimental spiders fell to 0.2 and showed little variation for the remainder of the experiment (Figure I.12).

A summary of the ion balance of the feeding spiders is presented in Table I.5. The combination of anal excretion and accumulation of ions within the spider accounts for the ingested K, within the limits of

Figure I.13      Effect of a single meal on the haemolymph Na and K concentration of *P. antipodiana*.    (a) Sodium concentration.    (b) Potassium concentration.    Horizontal bar represents feeding period.    Vertical bars =  $\pm 1$  S.E.M.    Open symbols, control spiders hydrated and starved,  $n = 8$ .    Closed symbols, experimental spiders, hydrated and fed three cockroach nymphs for 24 hours,  $n = 8$ .

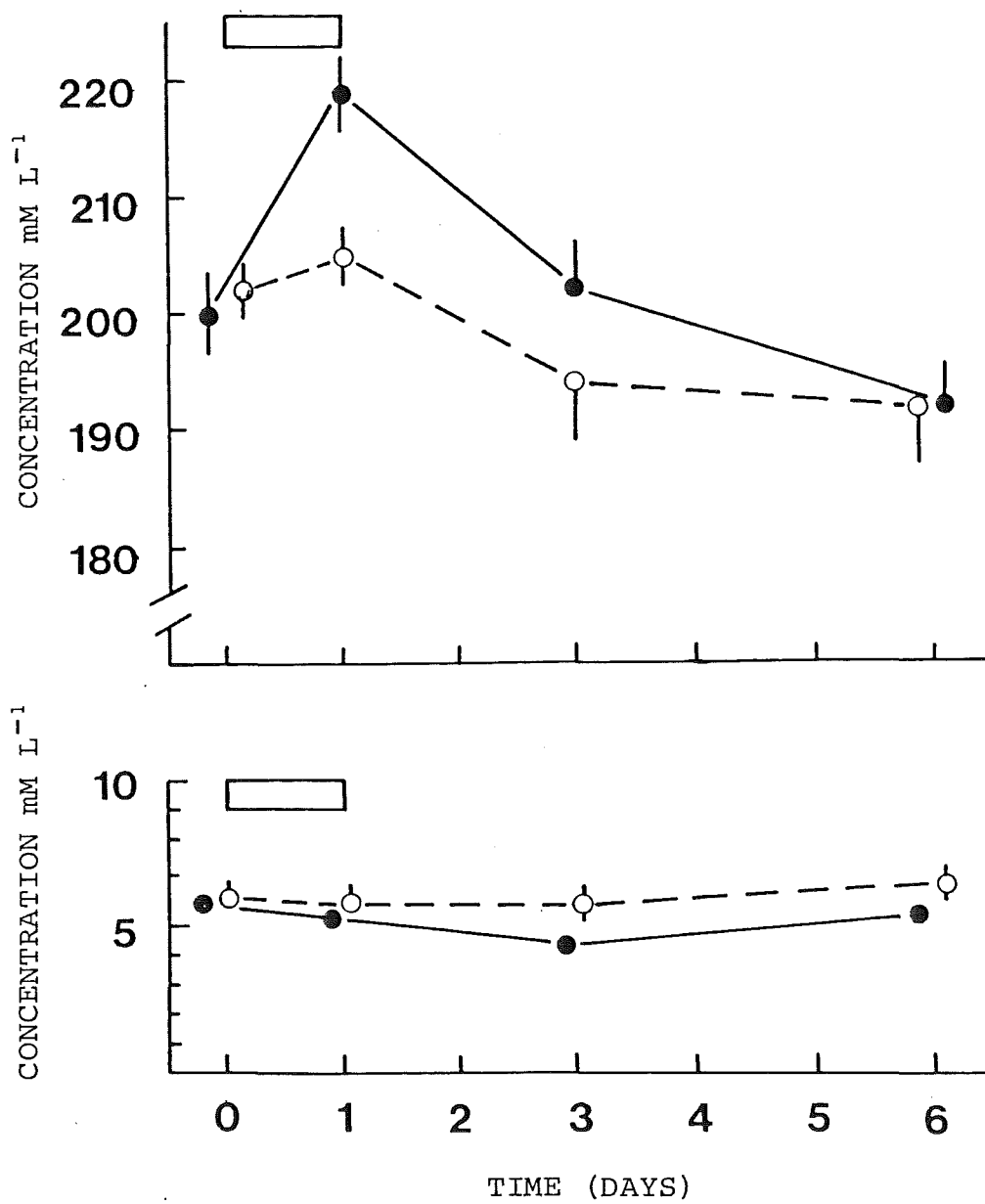


Table I.5     Ingestion and excretion of Na and K by spiders provided with a single meal of *P. americana* nymphs.  
Values = mean of eight spiders.

	A Estimated content of food	B Measured content of food debris	A-B Estimated ingestion	Anal excretion resulting from feeding†	Calculated content of starved spiders	Measured content of fed spiders 5 days after meal
Potassium μMoles	22.7	8.5	14.2	6.08	54.7	61.3
Sodium μMoles	10.7	8.2	2.5	1.6	77.4	79.6
Dry material mg	39.5	14.0	25.4	-	-	-

† Duration of anal excretion = six days.

the experiment. Of an available 22.7  $\mu\text{M}$  K, 14.2  $\mu\text{M}$  were ingested and 6.1  $\mu\text{M}$  excreted by the fed spiders, while the K content of the fed spiders increased by 6.6  $\mu\text{M}$ . With regard to Na, 2.5  $\mu\text{M}$  were ingested and 1.5  $\mu\text{M}$  excreted via the anus. Again there was an increase in the Na content of the fed spiders (2.2  $\mu\text{M}$ ) which, within the limits of the experiment, accounted for the difference in the amount of Na ingested and excreted. Therefore, it certainly does not appear necessary to invoke an extra anal excretory route to complete the Na balance sheet.

It is significant, however, that only 2.5  $\mu\text{M}$  of a total 10.7  $\mu\text{M}$  of Na available in the food were apparently ingested. This represents only 23% of the available Na, and is markedly less than the 62% of the available K and 65% of the available dry material which were ingested by the spiders in the same meal. This low percentage ingestion of Na relative to K resulted in an increased Na/K ratio of the food debris ( $1.03 \pm 0.13$ ,  $\bar{X} \pm \text{S.E.M.}$ ,  $n = 8$ ) when compared to the Na/K ratio of the whole food ( $0.46 \pm 0.02$ ,  $\bar{X} \pm \text{S.E.M.}$ ,  $n = 20$ ). Furthermore, the reduced Na/K ratio of the excreta of fed spiders (Figures I.9 and I.12) can be accounted for by the proportionately low relative ingestion of Na. Therefore, although it does not appear necessary to propose an extra anal excretory route to account for the overall Na and K balance, it still remains to be explained how ingestion of more than 60% of the K and other dry material of the prey were accompanied by only 23% of the available Na.

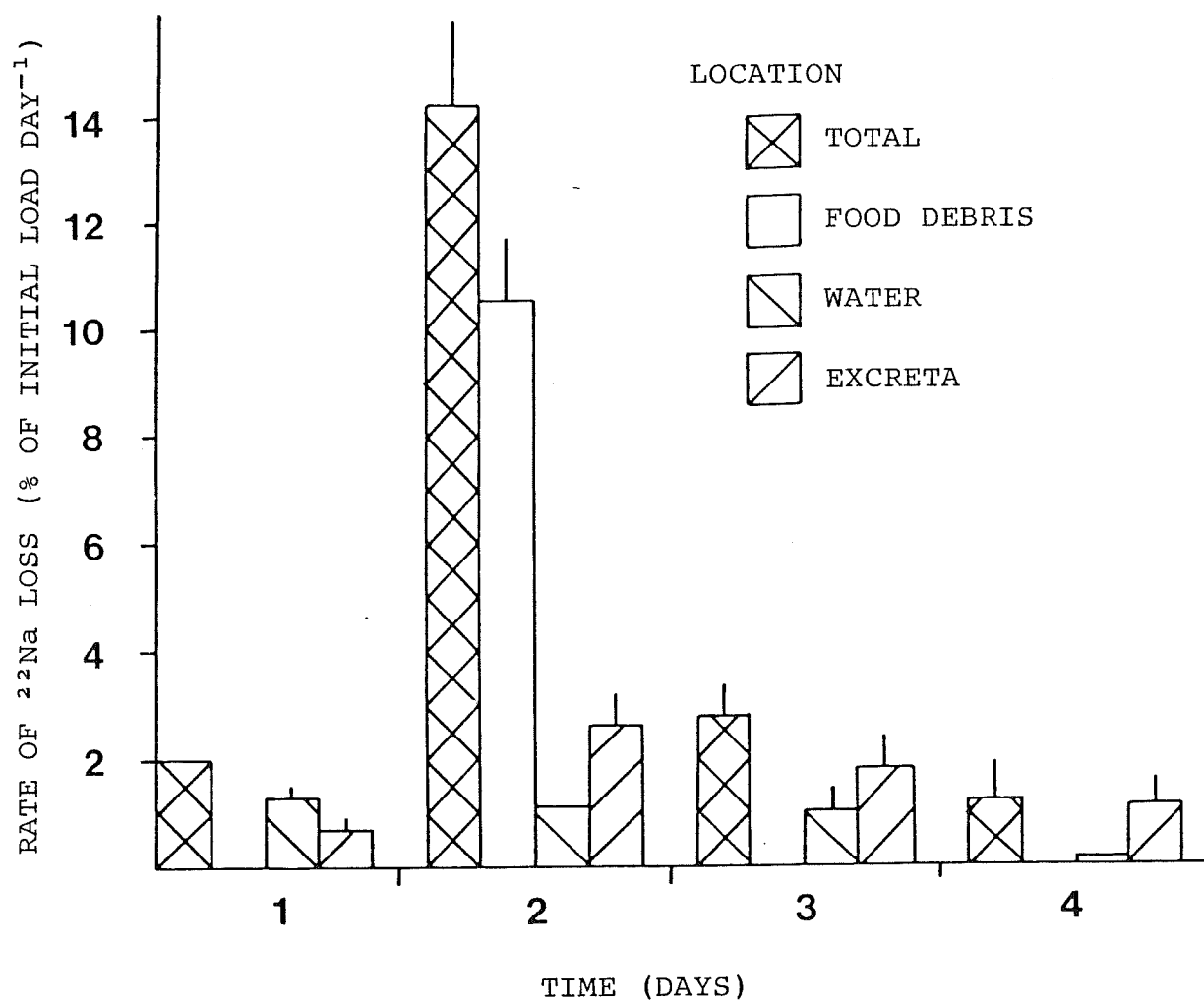
The proposed alternative explanation that feeding is a selective process was not supported by observations of feeding *P. antipodiana*. Hydrated spiders indiscriminately crushed prey items with their chelicerae and maxillary blades and it was not possible to identify any specific portion of the cockroach nymphs from the meal debris. Furthermore, sieving devices, which are present in the pre-oral region of spiders in general (Legendre, 1978), prevent the ingestion of large food particles, such as pieces of tissue, which in the cockroach are rich in K (Tucker, 1977a), but do not prevent the ingestion of the Na rich (Tucker, 1977a) haemolymph. Thus the sieving devices, if anything, would reduce K intake not Na.

### I.3.5 The Secretion of Na into the Prey

An alternative explanation for the low Na intake relative to K by feeding *P. antipodiana*, is that Na is secreted into the prey. The

Figure I.14     The routes of  $^{22}\text{Na}$  loss from *P. antipodiana* during and after feeding.     Horizontal bar represents feeding period. Vertical lines =  $\pm 1$  S.E.M.,  $n = 10$ .





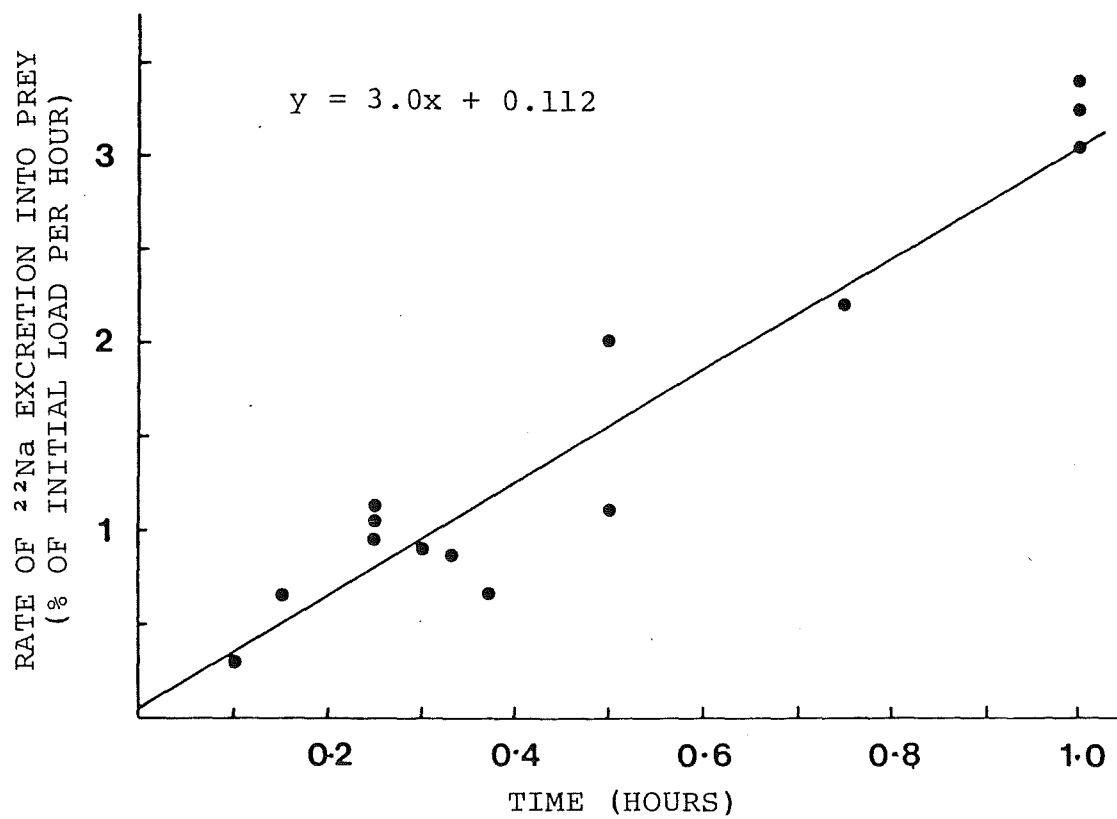
secretion of Na, by the spider, either into the prey during feeding or the prey debris after feeding, would result in an elevated Na content of the food debris and a low net ingestion of Na. The tick, *Dermacentor andersoni*, produces a Na rich salivary secretion which is injected into the host during feeding and accounts for 96% of the excreted Na (Kaufman and Phillips, 1973). Spiders are also known to secrete fluid into the prey during feeding. This fluid is thought to consist of copious quantities of digestive fluids produced by the midgut epithelium and glands located on the coxa of the pedipalps, and its function appears to be the extra oral digestion of the prey (Snow, 1970; Legendre, 1978).

To examine whether significant quantities of Na were secreted into the prey or prey debris, and subsequently lost in the debris, and to further partition the routes for Na loss, the Na pools of a number of spiders were labelled with  $^{22}\text{Na}$ . Spiders were injected with known amounts of  $^{22}\text{Na}$  and allowed 48 hours for recovery and equilibration. They were then transferred to experimental chambers lined with filter paper and provided with water. After a further 24 hours the spiders were transferred to new containers containing several cockroach nymphs plus water and they were allowed to feed for 24 hours before being transferred to clean containers with water only. For each 24 hour interval, the excreta, visualised under ultra violet light, were cut from the filter paper and their  $^{22}\text{Na}$  content measured. The remaining filter paper was inspected a second time, to confirm the absence of excreta, then subdivided into small squares and checked for  $^{22}\text{Na}$  activity. The  $^{22}\text{Na}$  activity of the cotton dental buds, in which the water was provided, was also determined and, after completion of feeding, the  $^{22}\text{Na}$  content of the food debris was measured.

The total rate of  $^{22}\text{Na}$  loss and its subdivision into that lost in either the food debris, water, or excreta is shown in Figure I.14.

In the day prior to feeding, 2% of the injected  $^{22}\text{Na}$  was lost from the spiders, all of this was located in either the water or the excreta. There were no regions of  $^{22}\text{Na}$  activity on the filter paper which were not associated with the excreta. During the day in which feeding occurred, the total loss of  $^{22}\text{Na}$  increased to 14.2%, but only 2.6% was present in the excreta and 1.1% in the water. The remaining 10.5% was located in the food debris. Following feeding, total rates of  $^{22}\text{Na}$  loss dropped to levels only slightly above prefeeding levels and, again, all was present in either the drinking water or the excreta.

Figure I.15     Rate of  $^{22}\text{Na}$  excretion into prey during feeding by  
*P. antipodiana*.     Each point represents an individual  
spider.



While the  $^{22}\text{Na}$  loss in the food debris is thought to result from secretion into the prey or debris by the spider, the loss in the water could result from the simple washing off of the dried salts which are commonly observed on the labium and maxilla of *P. antipodiana*, particularly those recently fed.

#### I.3.6 The Time Course of Na Loss into the Prey During Feeding

If the Na is secreted into the debris after feeding is complete, then this might be reasonably considered a rapid regulatory response (i.e., excretion) to excess Na taken in during feeding. However, if the Na is lost during the initial stages of feeding or continuously during feeding, then it is possible that the phenomenon could be considered as either an 'anticipatory regulation' or as incidental in some way to the feeding mechanism itself.

To determine the time course of Na secretion into the prey, spiders, labelled with  $^{22}\text{Na}$ , were fed individual cockroach nymphs and at various intervals feeding was interrupted and the  $^{22}\text{Na}$  content of the remains measured. The rate of  $^{22}\text{Na}$  excretion into the prey by spiders which were feeding continuously until interrupted is shown in Figure I.15. Each point represents the amount secreted by an individual spider. The secretion of  $^{22}\text{Na}$  into the prey during feeding appears to occur at a steady rate of 3% of injected  $^{22}\text{Na}$   $\text{hr}^{-1}$ . Furthermore, these experiments provided no evidence for a delay or, alternatively, an initial pulse of  $^{22}\text{Na}$  secretion into the prey.

#### I.4 DISCUSSION

The data presented indicate that hydrated adult female *P. antipodiana* excrete ions via two routes during and following a meal. During feeding, Na and presumably K are excreted continuously into the prey resulting in the loss of ions in the prey debris left upon completion of the meal. Following feeding, K, and to a lesser extent Na, is excreted via the anus during a diuretic period which lasts from 48 to 72 hours in the normally fed animal.

The significance of the two routes in the excretion of Na and K during and following a single meal is summarised in Table I.6.

Anal Na and K excretion were measured directly as described in the results. The amount of Na and K lost in the food debris was estimated assuming that similar proportions of the total Na, K and dry material were ingested with a meal. Within a prey item such as the cockroach, Na and K are partitioned into two different regions of the body; most of the Na in the haemolymph while the K is associated with the tissues (Tucker, 1977a). It is unlikely, however, that disproportionate ingestion of Na, K and dry material would occur during feeding, as *P. antipodiana* macerates the tissue extensively with the chelicerae and the maxillary blades and, like spiders in general (Snow, 1970; Mommsen, 1978), secretes powerful proteolytic enzymes into the prey which solubilise the tissue before ingestion.

Therefore, the expected Na and K content of the food debris, left on completion of the meal, can be calculated from the following relationship:

$$\text{Expected Na or K content of debris} = \left( \frac{\text{Total Na or K content of food}}{\text{Dry weight of food}} \right) \times \left( \frac{\text{Dry weight of food debris}}{\text{Dry weight of food provided}} \right)$$

The difference between the calculated and measured content of the food debris will represent the amounts of Na or K lost from the spider into the food debris. It should be noted that this represents only a part of the total Na or K excreted into the prey during feeding, as reingestion of excreted material appears unavoidable.

Table I.6 The routes of ionic excretion by *P. antipodiana* during and following a single meal. (Data calculated for ion balance experiment [Section I.3.2] presented in results.)

	Estimated total ingested †	Estimated total excreted (anal & coxal)	Estimated coxal excretion (24 hrs of meal)	Measured anal excretion associated with the meal ††
μMoles	14.7	7.4	1.3	6.1
Potassium				
% of total excretion	-	100	18	82
μMoles	7.0	6.4	4.8	1.6
Sodium				
% of total excretion	-	100	75	25

† Equivalent to 65% of the Na or K available in the food.

†† Anal excretion associated with the meal was calculated as the difference between the rate of Na and K excretion of fed animals and control starved animals during the meal and for 120 hours after the meal.

As a comparison the loss of Na into the food debris was also estimated by  $^{22}\text{Na}$ . The percentage of the total  $^{22}\text{Na}$  content of a labelled spider present in the prey debris on the completion of a meal will be approximately equal to the percentage loss of cold Na from the spider. The actual amount lost in  $\mu\text{Moles}$ , can be determined from the estimated Na content of the starved labelled spider before feeding. This will, in fact, give a conservative estimate of Na loss because, as feeding progresses, the specific activity of the  $^{22}\text{Na}$  will be reduced by the ingestion of cold Na from the prey.

Estimates of the Na loss in the prey debris by the two methods are in good agreement (Table I.6) and, even though they probably represent a conservative measure, Na loss via this route represents a major proportion of the total Na excreted.

Of the total Na and K ingested (65% of the Na and K available in the food), 90% and 50% were eliminated respectively. However, whereas 82% of the excreted K was eliminated via the anus, only 25% of the excreted Na was lost via this route. Conversely, 75% of the excreted Na and 18% of the excreted K was lost in the prey debris, a consequence of excretion into the prey during feeding.

Potential routes of excretion into the prey will be investigated and discussed in Section II. It should be noted, however, that the excretion of ions and water by systems other than the Malpighian tubules and rectum is common among the arachnids. The blood sucking gamasid mite, *Ornithonyssus bacoti*, excretes water from the salivary glands after feeding (Belozarov, 1958) and salivary glands are responsible for the excretion of water and ions by the ixodid ticks *Boophilus microplus* (Tatchell, 1967, 1969) and *Dermacentor andersoni* (Kaufman and Phillips, 1973). The coxal glands of the argasid tick, *Ornithodoros moubata*, function in volume and osmotic regulation, excreting ions and water during and after feeding (Lees, 1946; Kaufman *et al.*, 1981). However, the selective advantage of the use of two routes of excretion by *P. antipodiana* is not immediately obvious. In the tick, *D. andersoni*, Na and K excretion are also partitioned. Most of the Na is excreted into the prey during feeding by the salivary glands while the bulk of the K is excreted via the anus (Kaufman and Phillips, 1973). The relative sizes of the tick and the vertebrate host would ensure that little of the Na excreted into the host would be reingested by the tick, so that the salivary glands would function



as an efficient route for the excretion of Na and water. *P. antipodiana*, however, consumes prey which are generally smaller than itself and much of the material which is excreted into the prey is reingested as feeding continues (Section II), reducing the efficiency of this system as a means of Na and K excretion.

It is possible that as Na is lost constantly throughout feeding, its loss may be incidental to the feeding process, rather than a regulatory response, and the production of a K-rich Na-poor excreta simply a compensation for the low net ingestion of Na with the meal.

The source of the cations in the excreta is unknown. In *P. antipodiana*, and spiders in general (Figure I.1), the Malpighian tubules and stercoral pocket are encased in the midgut diverticula. Also, associated with the diverticula and filling most of the space between individual diverticulum, is the adipose tissue (Comstock, 1946). The complex arrangement of the diverticula prevents sampling of the Malpighian tubule fluid and midgut fluid making it difficult to determine directly the sources of material in the stercoral pocket. The faecal pellets, which constitute the bulk of the faecal material, are produced in the midgut. The loose faecal material passes from the diverticula to the gut lumen and is invested in the post diverticular midgut with what has been labelled improperly (Van der Borgh, 1966) a peritrophic membrane.

Kaufman and Phillips (1973) suggested that the Malpighian tubules were not involved in the production of fluid and ions excreted via the anus of the tick, *D. andersoni*. In this animal, 84% of the excreted K, but only 4% of the excreted Na, is voided via the anus. They proposed that the midgut of the tick transported Na, Cl and water into the haemolymph, but was relatively impermeable to K. Consequently, the K-rich material excreted via the anus of the tick was derived from the host blood passing directly from the midgut to the rectal sac.

The results presented here suggest that the midgut of *P. antipodiana* may also selectively absorb Na in excess of K, and therefore function in a similar manner as was proposed for the tick. During feeding there is an immediate loss of Na and K into the food which, in the absence of assimilation of either of the ions, would result in a reduction in haemolymph Na concentration of  $37 \text{ m mol l}^{-1}$  and haemolymph K by  $12 \text{ m mol l}^{-1}$ . However, the Na concentration of the haemolymph actually increased with

feeding while the K concentration decreased, although not to the extent that was predicted without assimilation (Figure I.7 and Section II). These changes occurred in spite of conditions in the gut favouring the absorption of K. The Na concentration of the food ( $105 \text{ m mol kg H}_2\text{O}^{-1}$ ) was markedly less than the haemolymph Na concentration ( $202 \text{ m mol l}^{-1}$ ), whereas the K concentration of the food ( $217 \text{ m mol kg H}_2\text{O}^{-1}$ ) was well in excess of the haemolymph K concentration ( $5.46 \text{ m mol l}^{-1}$ ).

An equally plausible explanation, involving assimilation of K into the haemolymph and subsequent excretion, can be proposed for both the tick and *P. antipodiana*. Potassium is the major cation excreted by the Malpighian tubules of the insects (Ramsay, 1953; Berridge, 1968; Irvine, 1969; Maddrell, 1969; Pilcher, 1970b) and is transported by most other active insect epithelia; salivary glands of *Calliphora* (Oschman and Berridge, 1970; Berridge *et al.*, 1975; Gupta *et al.*, 1978); the goblet cells in the gut of Lepidopterous larvae (Anderson and Harvey, 1966; Wood *et al.*, 1969; Blankemeyer and Harvey, 1978); the labial gland of adult *Antheraea pernyi* (Kafatos, 1968); the rectum of *Schistocerca gregaria* (Williams *et al.*, 1978); and the posterior rectum of the salt water mosquito larvae *Aedes taeniorhynchus* (Bradley and Phillips, 1975). Therefore, absorption of K by the midgut and rapid excretion by the Malpighian tubules or some other region of the gut would achieve the same results. In support of this it was demonstrated by Van Hook (1971) that 100% of the  $^{22}\text{Na}$ ,  $^{42}\text{K}$  and  $^{47}\text{Ca}$  ingested by the spider *Lycosa punctata* was assimilated.

As the Malpighian tubules of insects are such important excretory organs, it is generally felt that the Malpighian tubules in other groups, such as spiders and ticks, play a similar role. However, the Malpighian tubules of *P. antipodiana*, and spiders in general (Millot, 1949), are restricted to the tissue mass consisting of the abdominal diverticula and the storage tissue (Figure I.1). In this they differ markedly from the tubules of the insects which lie free in the haemolymph, and unless there is some means of circulating the haemolymph over the Malpighian tubules of spiders, they may not necessarily be involved in osmoregulation as they are in the insects.

It is possible that regions of the mid and hindgut could be active in excretion. Regions of the gut have been implicated in the excretion of ions and water in a number of arthropods. Mantel (1968) suggested

that the foregut of the terrestrial crab *Gecarcinus lateralis* may function in the excretion of ions and water during the moult cycle. The midgut of Lepidopterous larvae has been shown to excrete K (Harvey and Nedergaard, 1964; Harvey and Zerahn, 1969; Blankemeyer and Harvey, 1978) and Harvey and Zerahn (1972) have proposed that the K pump *in vivo* can be considered as an accessory excretory organ which, in these plant-eating insects whose diet has a rich K content, relieves the Malpighian tubules and rectum from one of their normal functions, regulating the K concentration of the haemolymph. The posterior rectum of the saltwater mosquito larvae *A. taeniorhynchus* secretes a hyperosmotic fluid which is responsible for much of the osmotic and ionic regulation of the haemolymph of these insects (Bradley and Phillips, 1975, 1977a, b).

Spiders are thought of as fluid feeders, the prey being partially digested in the pre-oral cavity by the enzymes contained in the fluid regurgitated through the mouth (Meglistch, 1972). In many spiders it is felt that the water available in the prey is sufficient to meet the needs of the animal (Comstock, 1940). With this in mind, it was of interest to determine the relative importance of the prey water in the overall water balance of *P. antipodiana*.

Feeding hydrated *P. antipodiana* had two sources of water, that in the food and the free water provided; absorption of water from the atmosphere does not occur (Section III). Water loss occurs through excretion, of which there are two components, anal excretion and excretion into the prey, and transpiration. Each of the components of the water balance can be determined for the ion balance experiment described in the results, and are summarised in Table I.7. The mean total water available to the spiders in the prey was 142 mg (weight of the food provided  $\times$  the relative water content of the food). However, the proportion of the total water that could be ingested is unlikely to exceed the proportion of the total dry material ingested (65%). Therefore, at the most 92 mg of water would be ingested and, in fact, it is likely to be less as evaporation of water from the macerated prey tissue in the pre-oral cavity would occur during feeding (Section IV).

Transpirational water loss can be estimated from rates of water loss observed in spiders maintained under similar conditions without food or water (Section IV) and is equivalent to  $19 \text{ mg day}^{-1}$  or a total of 114 mg for the six day period involving feeding and diuresis. During the same

Table I.7 The water balance of hydrated *P. antipodiana* provided with a single meal and free water. (Data calculated for ion balance experiment from Section I.3.2.)

	Calculated total available in food	Calculated maximum possible ingestion	Calculated transpirational loss over six days	Measured anal excretion over six days†	Calculated coxal excretion limited to feeding period †	Total loss
Water (mg)	142	92	114	93	37	244
Percent of total water ingestion from prey	-	100%	123%	101%	40%	265%

† Assumed 1  $\mu$ l of urine or coxal fluid equivalent to 1 mg of water.

period, a total of 93 mg of urine were excreted via the anus (Figure I.5).

Coxal excretion is responsible for the loss of Na and K in the prey debris and knowing the composition of the coxal fluid (Section II) and the amount of Na and K lost in the debris, the volume of coxal fluid (37  $\mu$ l) that would excrete these quantities of cations can be calculated.

From Table I.7 it can be seen that the amount of water obtained from the prey was small relative to the losses the spider experienced during feeding and diuresis. Transpirational and excretory water loss during this period were two to three times the maximum amount that could be obtained from the prey. Allowing for increases in the dry weight of the spider, the difference between the amount of water gained from the prey and the amount lost during and following feeding was equivalent to 22% of the body weight of the spiders. But in the six days following feeding, the spiders only lost 2-4% of their body weight, thus the spiders must drink following feeding and this water was of considerable importance to the water balance of the spider.

Hydrated *P. antipodiana* experience a period of diuresis following a meal and, in view of the small amount of water obtained from the meal, the nature of the diuresis is of some interest. Post prandial diuresis has been noted in a number of arthropods. In blood sucking arthropods such as the mosquito (Stobbs, 1977); *Rhodnius prolixus* (Wigglesworth, 1931; Maddrell, 1964); *D. andersoni* (Kaufman and Phillips, 1973); *Glossina austeni* and *G. morsitans* (Gee, 1975a,b, 1976); and *O. moubata* (Kaufman *et al.*, 1981) an extensive post prandial diuresis is associated with the excretion of excess water and ions ingested with a meal. The primary function of the diuresis being a reduction in body volume (Maddrell, 1964; Gee, 1974; Stobbs, 1977). Less dramatic diuretic responses following feeding have been observed in the stick insect *Carausius morosus* (Pilcher, 1970a) and the cotton stainer *Dysdercus fasciatus* (Berridge, 1965a). Berridge (1965b) argued that the diuresis exhibited by *D. fasciatus* resulted from the need to remove excess ions ingested with the meal rather than a need to reduce body volume. In water-fed *S. gregaria* small volumes of rectal fluid of low ionic concentration are produced, whereas saline-fed locusts produce large volumes of urine of high ionic content (Phillips, 1964). In this latter case, the need to remove the excess inorganic ions, in effect, converts the animal into a fluid excreting insect. A similar diuretic excretory response occurs in

*P. antipodiana*. The increase in size of the animal with the meal (11%) is of little significance to the animal and, as already noted, the volume of fluid ingested with the meal is less than the transpirational loss experienced over the feeding and diuretic periods. However, the diuresis which is maintained by drinking (Section IV) increased in both magnitude and duration when the salt load in the food is increased (Section II).

The diuretic response of *D. fasciatus* was intensified through the inability of the animal to produce a hyperosmotic urine (Berridge, 1965b). The urine produced by both fed and starved *P. antipodiana* was only slightly hypo-osmotic to the haemolymph, but it was markedly hypo-ionic, the ions Na, K and Cl contributing less than 50% of the total osmotic pressure of the urine. The hypo-ionic nature of the urine would, in effect, increase the volume required to excrete the excess ions ingested with a meal. Furthermore, *P. antipodiana* cannot compensate for this by increasing the osmotic pressure of the urine as even under conditions of severe dehydration the animal is incapable of producing a hyper-osmotic urine (Section IV).

Hypo-ionic urine has been noted in a number of arthropods. The urine of the aquatic larvae of *Sialis lutaria* is markedly hypo-ionic and it has been demonstrated that the osmotic pressure difference is generated by ammonium bicarbonate (Shaw, 1955). Terrestrial animals, for reasons of water economy, are generally not ammoniotelic (Edney, 1977), although there are exceptions among the insects; the cockroach *P. americana* (Mullins, 1974; Mullins and Cochran, 1974) and the larvae of the blowfly *Sarcophaga bullata* (Prusch, 1972). Spiders are recognised as guanotelic (Anderson, 1966). Guanine, because of its low solubility, contributes little to the osmotic pressure of the urine. Paper chromatograms of the stercoral fluid of *P. antipodiana* confirm the presence of guanine, but not xanthine, hypo-xanthine, uric acid or urea, suggesting that *P. antipodiana* is guanotelic. However, other nitrogenous components such as amino acids and creatine may contribute to the osmotic pressure of the stercoral fluid. Phillips (1964b) noted the discrepancy between the total concentration of Na, K and Cl in the rectal fluid of *S. gregaria* and the osmotic pressure of the fluid, and suggested that organic solutes such as these and inorganic ions other than Na, K and Cl were responsible for the difference.

## I.5 SUMMARY

1. The total ion and water balance of the spider *Porrhothele antipodiana* was studied.
2. *P. antipodiana* excretes a fluid volume equivalent to more than 100% of the total water gained from a meal. The excretory water loss occurs as a result of excretion into the prey during feeding and an anal diuresis following the meal.
3. Transpirational losses during feeding and the diuretic period are equivalent to 123% of the water gained from the prey. Total losses (excretory plus transpirational) are in excess of twice the volume of fluid obtained from the prey, and *P. antipodiana* drinks free water to maintain its water balance.
4. Two routes of salt excretion are utilised by *P. antipodiana* to eliminate excess salts ingested in the meal.
5. A Na rich fluid is excreted into the prey at a constant rate throughout feeding. A K rich fluid is produced by the anal system in the diuretic period following the meal.
6. The anal system produces (volumes of) fluid at all times in the hydrated animal. Excretion by the second route was limited to periods of feeding.
7. Of the total Na and K excreted 25 and 82% respectively are lost via the anus. The remainder in each case is excreted into the prey during feeding.
8. The fluid produced by the anal system was in starved animals slightly hypo-osmotic and in fed animals iso-osmotic to the haemolymph. It was at all times markedly hypo-ionic with respect to the ions, Na, K and Cl.

II. SALT EXCRETION IN *PORRHOTHELE ANTIPODIANA*. THE ROLE OF THE  
COXAL GLANDS AND THE ANAL SYSTEM.



## II.1 INTRODUCTION

*Porrhothele antipodiana* excretes Na and K into the prey during feeding, resulting in a loss of Na and K in the prey debris left on completion of the meal (Section I). There are several possible sources of excretion into the prey. The midgut diverticula provide copious quantities of digestive fluid which are regurgitated over the prey via the mouth (Millot, 1949; Legendre, 1978). The ganthocoxal acini also produce digestive secretions which are excreted into the prey (Snow, 1970), and varying amounts of poison are secreted into the prey by spiders (Millot, 1949).

To investigate possible involvement of these glands in the excretion of Na and K into the prey, the fluids produced by these glands were tested to determine whether they satisfied the following criteria (Section I).

- (i) Excretion was restricted to and occurred continuously throughout feeding.
- (ii) Excretion of Na into the prey exceeds K at a ratio of 3.7 : 1.
- (iii) As excretion into the prey occurs during feeding, reingestion of excreted material appears unavoidable. Therefore the rate of Na and K excretion must be sufficient to account for the loss in the prey debris and reingestion.

Quite unexpectedly it was found that the coxal glands also excrete fluid into the prey during feeding and that this excretion appears responsible for the Na and K loss in the prey debris.

In this section coxal excretion in hydrated adult female *P. antipodiana* is described and the operation of the coxal glands as excretory organs tested, and compared to the response of the anal system, by modifying the ionic load that *P. antipodiana* has to handle during and following feeding. Finally, a possible function of the coxal glands in *P. antipodiana*, and spiders in general, is proposed.

## II.2 METHODS AND MATERIALS

Adult female *P. antipodiana* collected from Whalers Bay and Third Bay, Kaikoura, New Zealand, and maintained in the laboratory as previously described, were used in all experiments.

### II.2.1 Collection of Coxal Fluid

Coxal fluid was collected directly from the openings of the coxal glands. The spiders were lightly anaesthetised with  $\text{CO}_2$ , mounted dorsal side down over a small plasticine ridge, and the walking legs positioned to expose one of the anterior coxal openings located between walking legs one and two. The hairs extending laterally from the edge of the sternum towards the legs were shaved off to expose the groove leading from the coxal opening forward to the pre-oral region. This groove was blocked between the pre-oral region and the coxal opening to prevent contamination of coxal fluid with food material, and also between the anterior and posterior coxal openings to prevent the collection of coxal fluid excreted from the posterior opening. Each blockage consisted of a thin base of "Supa Glue" (Selleys Chemicals) upon which a small dam was constructed by melting small pieces of dental wax with a heated wire. The "Supa Glue" was required because the dental wax would not adhere to the cuticle of the cephalothorax.

At the completion of the collection of the coxal fluid the blockages were tested for leakage. Drops of concentrated amaranth solution were placed either in the pre-oral region or in the coxal groove behind the posterior blockage and suction applied with a small capillary pipette placed in the coxal groove at the base of the blockages. If amaranth-stained fluid was collected, the coxal fluid obtained from that spider was rejected as contaminated.

Two grooves are present, one on either side of the sternum. In all spiders, except those injected with inulin, only one groove was blocked. In inulin-injected spiders both grooves were blocked to prevent the ingestion of inulin, and artificial coxal fluid ( $\text{NaCl}$ ,  $120 \text{ mM l}^{-1}$ ;  $\text{KCl}$ ,  $30 \text{ mM l}^{-1}$ ) was applied to the food in the pre-oral region at a rate of  $1 \mu\text{l min}^{-1}$ .

Spiders were allowed 30-60 minutes recovery after completion of the blockages and then provided with the soft abdominal contents of a freshly

killed cockroach nymph. Generally, hydrated spiders would begin feeding immediately, however, at times feeding had to be initiated by stimulating the chelicerae of the spider with forceps. Throughout feeding the opening of the coxal gland was observed under the binocular microscope. Lighting was provided by a Schott fibre optics cold light source.

Fluid emerging from the coxal openings was collected with a small silicone-coated pyrex capillary. Before collection a small amount of liquid paraffin was drawn into the capillary to prevent the evaporation of collected fluid. The coxal fluid was stored briefly under liquid paraffin before analysis.

The volume of coxal fluid collected was measured by drawing the fluid into 10  $\mu$ l silicone-coated microcaps (Drummond Ltd.). When the coxal fluid did not fill the microcap, fluid volume was calculated by simple proportion comparing the length of the microcap filled with fluid to the total length of the microcap and assuming the bore of the microcap was constant.

## II.2.2 Collection of Haemolymph and Stercoral Fluid

Both haemolymph and stercoral fluid were collected in the same manner as described previously (Section I.2.5).

## II.2.3 Injection Procedures and Counting of Isotopes

$^{22}\text{Na}$  and ( $^3\text{H}$ )-inulin were obtained from New England Nuclear. The stock  $^{22}\text{Na}$  was diluted with saline (composition given in Section I.2.3) and injected directly into the third walking leg as described previously (Section I.2.3) at a concentration of 0.074 MBq per gm spider. Inulin was diluted in distilled water and injected by the same method 90 minutes before the spider was fed. Sufficient inulin was injected to ensure an initial concentration of 3,500 dpm  $\mu\text{l}^{-1}$  haemolymph.

The injection of NaCl into the spiders for Na loading was carried out in the same manner as  $^{22}\text{Na}$  injection.

The ( $^3\text{H}$ )-inulin activities of both coxal fluid and the haemolymph were measured in either a Unilux II or Phillips PW4540 liquid scintillation counter. One microlitre of either haemolymph or coxal fluid was added to

1 ml of glass distilled water in a clean scintillation vial. Ten mls of scintillation cocktail (2 parts toluene : 1 part Triton X, 5 gm of PPO  $l^{-1}$ ) were added and the whole mixed thoroughly. Vials were temperature and light adapted in the counter for at least 12 hours before counting for 10 minutes. Quenching was estimated by the channels ratio technique using a series of commercially prepared quenched tritium standards (Amersham Searle Corp.).

The  $^{22}Na$  activity of the filter paper, cotton dental buds and food debris was measured in an Ortec gamma well counter as previously described (Section I.2.3).

#### II.2.4 Chemical Analysis

The osmotic pressure, Na and K concentration of the haemolymph and stercoral fluid were measured as described previously (Section I.2.6). Coxal fluid was diluted with appropriate volumes of glass distilled water and the Na and K concentration measured against NaCl and KCl made up in distilled water with a Varian 1200 Atomic Absorption spectrophotometer. The osmotic pressure of the coxal fluid was measured by the method of Ramsay and Brown (1955) with the modifications noted in Section I.2.6.

#### II.2.5 Anatomical Techniques

Spiders were killed with ether, the abdomen removed and the walking legs cut off at the junction of the coxa and the trochanter. The cephalothorax was then fixed in alcoholic Bouins for 48 hours and stored in 70% ethanol. Before embedding, the tissues were dehydrated for 12 hours in Murray's dehydrator (equal volumes of absolute ethanol, chloroform and glacial acetic acid saturated with  $HgCl_2$ ) then soaked for 24 hours in Murray's softener (equal volumes of phenol and chloral hydrate warmed to form an oily liquid at room temperature). The tissue was then double embedded in 1% celloidin and paraffin wax and sectioned at 8-10  $\mu m$  on a Beck microtome. Sections were mounted on glass slides and stained with either Ehrlich haemotoxylin or Mallory triple stain.

#### II.2.6 Paper Chromatography

Samples of coxal fluid were spotted onto Whatman No. 1 Chromatography

paper and run in Shandon Unikit chromatography tanks in the following solvent systems:

- (1) Descending single dimension chromatography -
  - (a) 70% n-Propanol
  - (b) n-Butanol (12 parts) : Acetic acid (3) : Water (1)
  - (c) The upper phase of the following solution, n-Butanol (4 parts) : Acetic acid (1) : Water (5)
- (2) Ascending two dimensional chromatography -
 

The first direction, Isopropanol (170 ml), concentrated HCl (41 ml) and distilled water (39 ml).

Second direction, n-Butanol saturated with water (100 ml) and 15M  $\text{NH}_4\text{OH}$  (1 ml) (Wyatt, 1955).

The chambers were saturated with the solvent systems overnight then the chromatograms run until the solvent front was within 1 cm of the end of the paper.

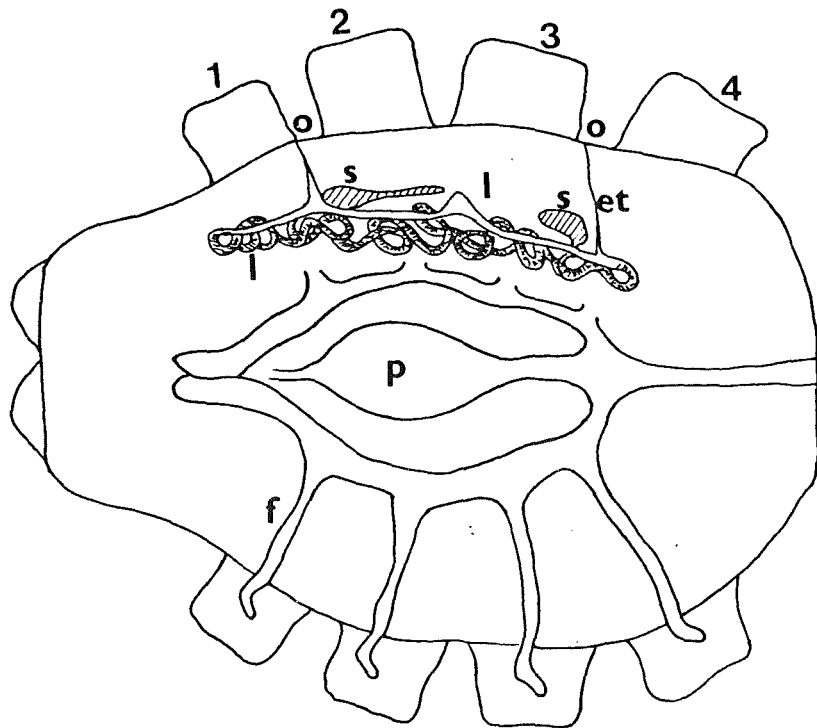
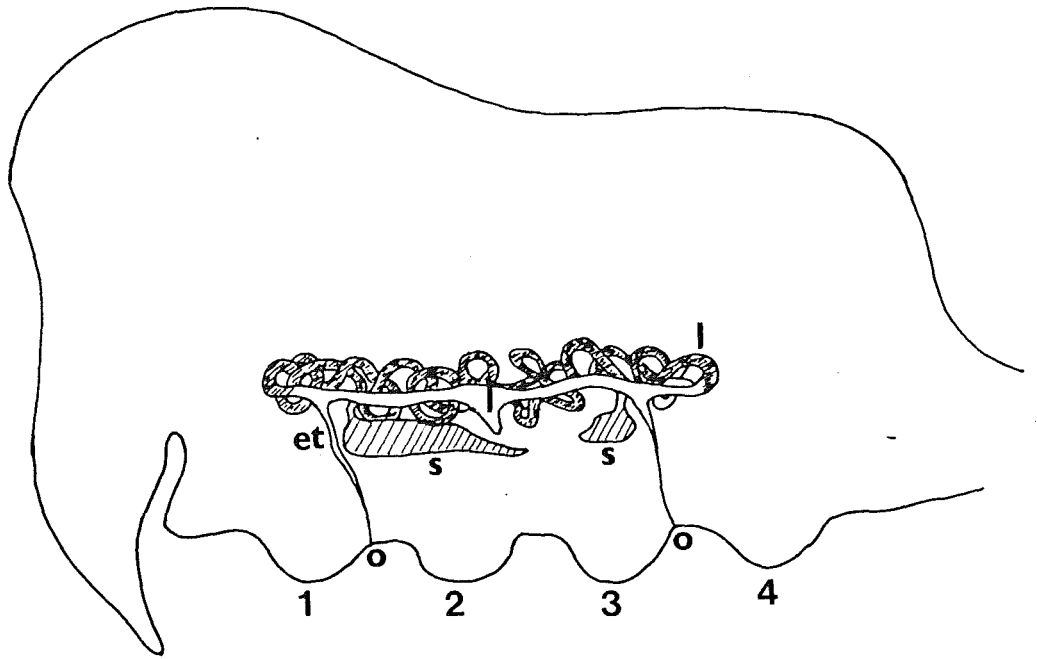
Standard  $5 \text{ mM l}^{-1}$  solutions of guanine, uric acid, xanthine, hypoxanthine and urea were spotted onto chromatography paper and run at the same time as the coxal fluid. Guanine, xanthine, hypoxanthine and uric acid were visualised with U.V. light. Urea was developed with Ehrlich reagent [10% p-Dimethylaminobenzaldehyde in concentrated HCl (10 ml) mixed just before use with acetone (40 ml)].

## II.2.7 Sample Preparation for the Scanning Electron Microscope

The cephalothoraxes of freshly killed spiders were mounted dorsal surface down on a glass rod. This was necessary to prevent the coxal segments of the legs collapsing against the sternum of the cephalothorax and covering the coxal groove.

The tissue was then fixed in 2% gluteraldehyde in cacodylate buffer, at  $4^\circ\text{C}$ , for 6 hours, washed overnight in buffer and then post-fixed in 1%  $\text{OsO}_4$  for 6 hours to ensure hardening. Fixed tissue was dehydrated through an alcohol series, infiltrated with amyl acetate and dried in a critical point drier. Specimens were mounted on stubs with "5 Minute Araldite" (Selleys Chemicals) coated with a 50 nm thick layer of gold (Polaron Equipment, E5000 SEM Coating Unit) and viewed with a Cambridge Stereoscan 600 scanning electron microscope.

Figure II.1 The general arrangement and positioning of the coxal glands in the cephalothorax of *P. antipodiana*. s = saccule; l = labyrinth; o = opening to the coxal glands; p = pumping stomach; f = foregut diverticula; et = exit tubule.



### II.3 RESULTS - ROUTES OF FLUID SECRETION INTO THE PREY

Observation of restrained feeding spiders confirmed that significant quantities of fluid were secreted by *P. antipodiana* into and over the prey during feeding. The fluid consisted of at least two components, one regurgitated via the mouth, the other excreted by the coxal glands.

#### II.3.1 Regurgitated Fluid

The gut contents of spiders were stained red by providing them with an 8 mM l<sup>-1</sup> amaranth solution for drinking rather than tapwater. When these spiders were fed, fluid, recognisable as gut fluid because of its red coloration, was regurgitated over the prey. The coxal fluid of these animals did not contain any amaranth. Regurgitation of the fluid was not continuous; its first appearance coincided with prey capture and subsequently brief periods of fluid regurgitation, during which the prey and pre-oral region were flooded, were interspersed with longer periods of suction by the pumping stomach and ingestion of suspended food particles.

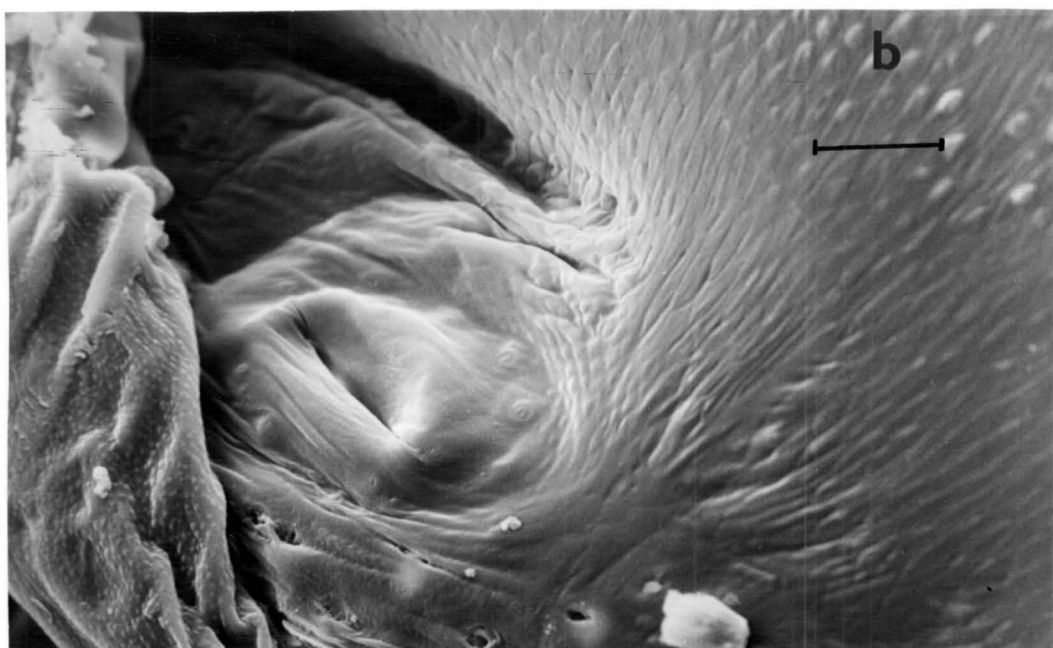
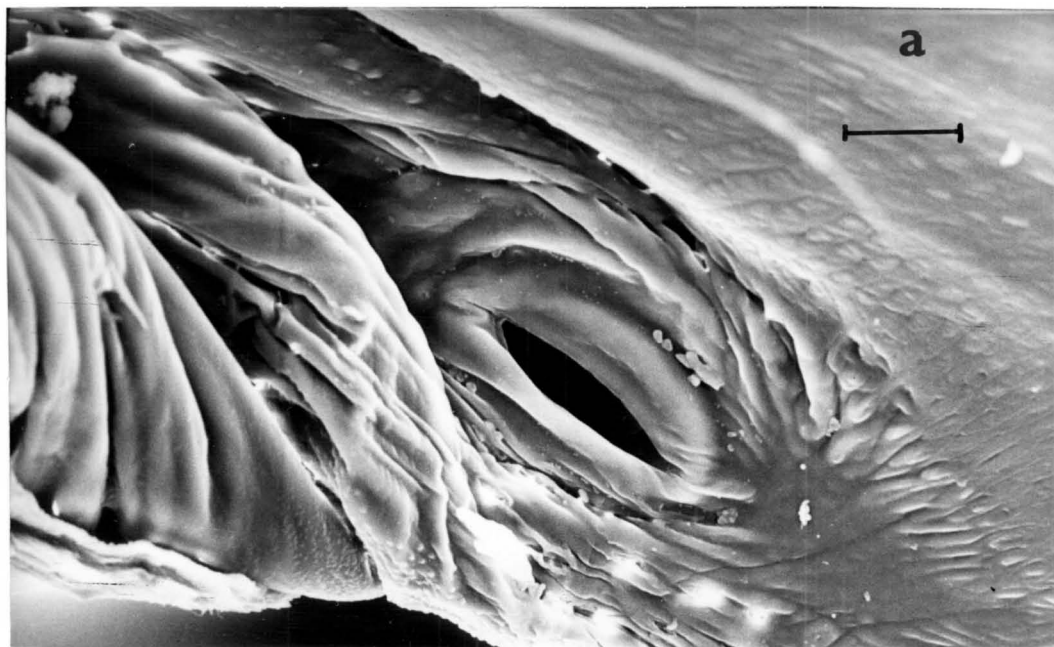
Collection of regurgitated fluid, which was not contaminated by partially digested food material, was only possible from the initial regurgitation. The coxal grooves were blocked in the normal manner and spiders enticed to feed on pieces of cleaned cuticle taken from freshly killed cockroaches. The regurgitated fluid, collected in this manner, was opaque, noticeably viscous in appearance and had a Na concentration of 128.5 mM l<sup>-1</sup>, slightly in excess of the K concentration of 101.5 mM l<sup>-1</sup> (Table II.1).

Table II.1 The cation composition of the initial fluid regurgitated via the mouth of *P. antipodiana* when stimulated to feed on pieces of cleaned cockroach cuticle. ( $\bar{X} \pm \text{S.E.M.}$ ,  $n = 5$ )

Regurgitated fluid (mM l <sup>-1</sup> )	
Na	128.5 $\pm$ 12.1
K	101.5 $\pm$ 10.0
Na/K	1.3 $\pm$ 0.18



Figure II.2    Scanning electron micrograph of the coxal opening on the posterior aspect of walking leg one.    (a) Coxal opening open as when the coxal gland is excreting.    (b) Coxal gland closed as between periods of excretion.



### II.3.2 Coxal Excretion

The coxal glands of *P. antipodiana* are located on the lateral edges of the cephalothorax. Their general arrangement conforms to that described for the mygalomorph spiders by Buxton (1913) (Figure II.1). The gland consists of two saccules, located opposite walking legs one and three, which communicate with a coiled labyrinth tubule. The tubular labyrinth extends from the base of the pedipalps posteriorly to the posterior aspect of walking leg four. The complex labyrinth, which appears to be common to both saccules, exits via two openings. The openings are located in the junction between the posterior aspect of the coxa of walking legs one and three and the soft arthrodial cuticle which links the coxa of the legs to sternum (Figure II.2a). The openings to the coxal glands can be closed and are drawn into a tight slit when closed between periods of excretion (Figure II.2b).

Coxal fluid emerges from the coxal opening at a constant basal rate. Superimposed on this is a series of pulses which generally coincide with regurgitation of fluid through the mouth.

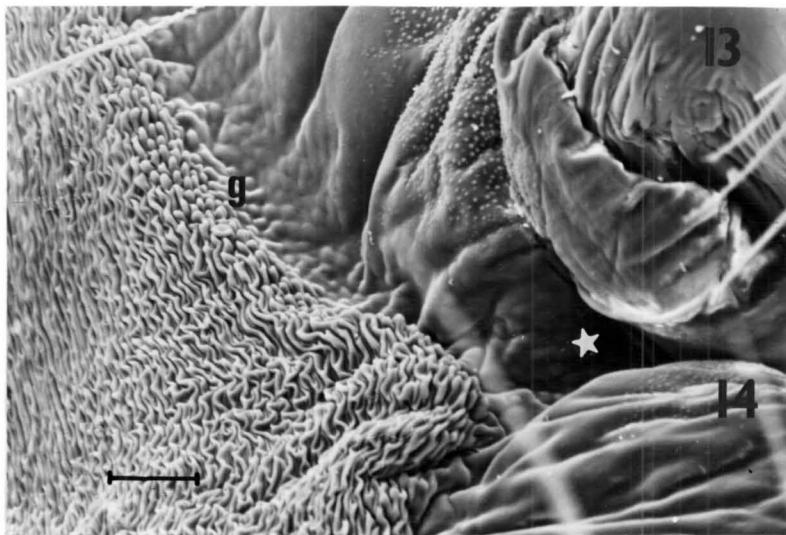
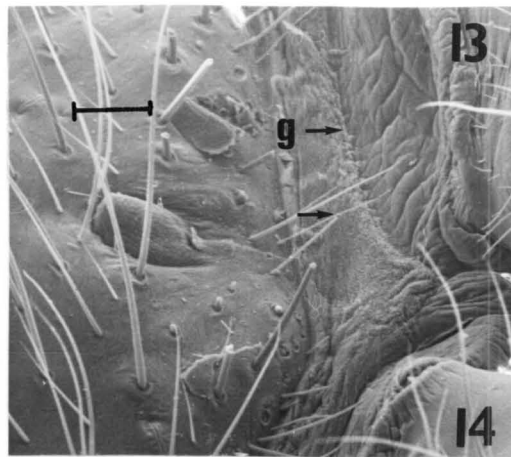
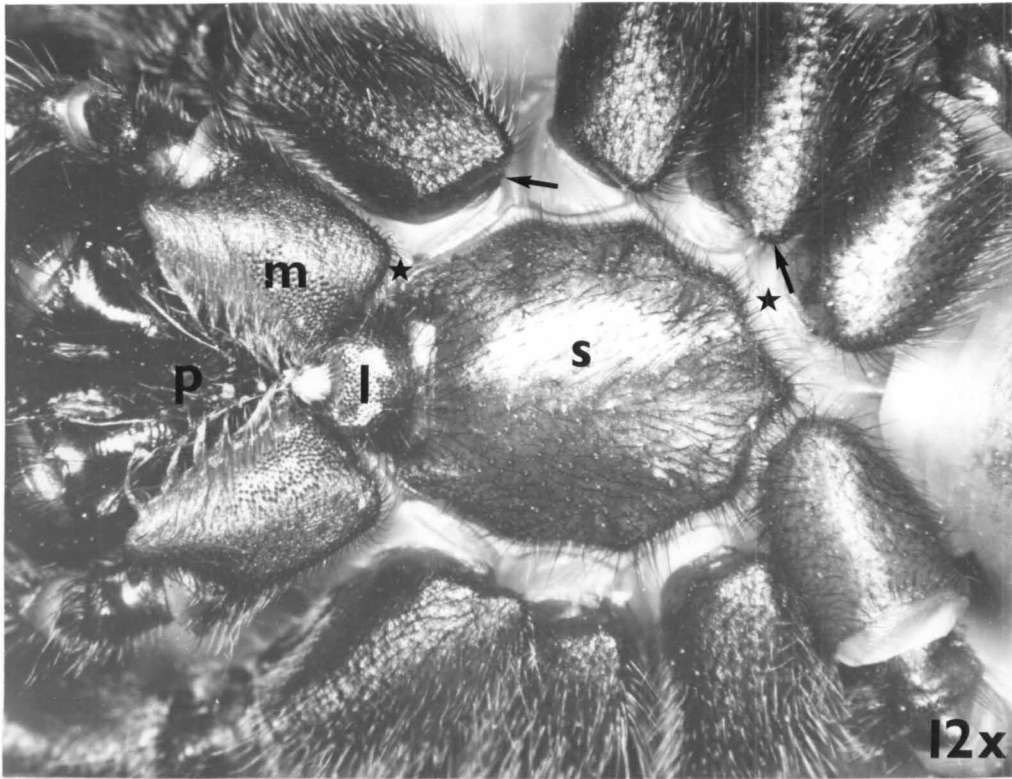
The fluid excreted by the glands does not accumulate in the region of the openings but is transported anteriorly to the mouth along a cuticular groove on the ventral surface of the cephalothorax (Figure II.3). The groove is located in the soft arthrodial cuticle that links the sternum and coxa of the walking legs. It runs parallel to the edge of the sternum, extending from opposite the leading edge of walking leg four forward to the gap between the maxilla and labium. The groove bends laterally towards the posterior aspect of each walking leg (Figure II.4a) and, in the case of legs one and three, this brings it into close proximity with the coxal openings (Figure II.4b). Consequently, fluid emerging from the coxal openings readily flows into the coxal groove, along which it is transported to the pre-oral region under the combined effects of suction from the mouth and continued excretion by the coxal glands.

The cuticle within the groove differs in appearance to that surrounding it (Figure II.5). The arthrodial cuticle on the sternal side of the groove has a striated appearance due to closely packed folds or ridges of cuticle which run parallel to the groove (Figure II.6). On the other side of the groove (the coxal side), the cuticle is much smoother in appearance with broader ridges and folds which run perpendicular to the groove (Figure II.7). The surface has a spotted appearance due to the

Figure II.3 The ventral surface of the cephalothorax of *P. antipodiana* showing the coxal groove (between the asterisks) running from opposite the anterior aspect of walking leg 4 forward to the gap between the maxilla and the labium. The position of the coxal openings indicated by arrows. l = labium; s = sternum; m = maxilla; o = coxal openings; p = pre-oral region.

Figure II.4a Scanning electron micrograph of the coxal groove at the junction of walking legs 3 and 4, showing the way in which the coxal groove bends laterally towards the posterior aspect of leg 3. Note also that the coxal groove does not extend beyond the anterior aspect of leg 4. Scale bar = 200  $\mu$ . g = groove; L3 = coxal segment of walking leg 3; L4 = coxal segment of walking leg 4.

Figure II.4b Scanning electron micrograph showing close proximity of the coxal opening (\*) on walking leg 3 to the start of the coxal groove at the posterior aspect of walking leg 3. Scale bar = 40  $\mu$ . \* = coxal opening; g = groove; L3 = coxal segment of walking leg 3; L4 = coxal segment of walking leg 4.



presence of many closely packed small protuberances. The coxal groove has a similar appearance to that of the cuticle on the coxal side, except the small protuberances are absent (Figure II.8).

The cuticle within the groove appears to be hydrophilic, as fluid placed in the groove spreads along its length. Similar fluid placed on the cephalothoracic cuticle remains in discrete drops.

Fluid excretion by the coxal glands of hydrated restrained spiders was restricted to periods of feeding. In the pre-oral region the coxal fluid flooded the prey, combining with the regurgitated midgut fluid to suspend the partially digested food material. Much of the coxal fluid was ingested with the food, however, at the completion of the meal the debris consisted of a very moist paste. The bulk of the fluid content of the paste coming from the regurgitated fluid and coxal fluid.

#### II.3.2.a Composition of coxal fluid

Unrestrained spiders required up to three hours to ingest prey items. However, during this time they did not feed continuously, often leaving the prey to carry out web construction, grooming or capture further prey. Spiders, restrained as described in the methods, would seldom feed continuously for periods in excess of 50 minutes. Listed in Table II.2 is the mean rate of excretion and composition of fluid excreted from one of the anterior coxal openings of spiders fed continuously for forty minutes. Also shown is the composition of the haemolymph before and after feeding.

The collected coxal fluid was transparent with no obvious pigmentation or suspended material present in it. The protein content as measured by the Folin Lowry Method was low ( $0.6 \mu\text{g}$  protein per  $\mu\text{l}$  coxal fluid), as was the total amino acid content. Paper chromatograms of the coxal fluid, run in a number of solvents, failed to demonstrate the presence of urea, uric acid, guanine, hypoxanthine or xanthine.

Quantitative collection of coxal fluid from more than one opening at any one time was not possible. However, coxal fluid was always excreted from the anterior and posterior openings and the rate of excretion measured from the posterior coxal openings of three spiders,  $16.9 \mu\text{l hr}^{-1}$  coxal opening $^{-1}\text{gm}^{-1}$ , was not markedly different from the mean rate of  $18.2 \mu\text{l hr}^{-1}$  coxal opening $^{-1}\text{gm}^{-1}$  observed for the anterior openings.

Figure II.5 Scanning electron micrograph of the coxal groove showing the difference between the cuticle of the coxal groove and the surrounding cuticle. Scale bar = 40  $\mu$ .  
g = coxal groove; s = arthrodial cuticle linking the sternum and the coxal groove; c = cuticle linking the coxal groove and the coxal segments of the walking legs.

Figure II.6 Scanning electron micrograph of the arthrodial cuticle linking the coxal groove and the sternum. Scale bar = 10  $\mu$ .

Figure II.7 Scanning electron micrograph showing the cuticle which links the coxal groove and the coxal segments of the walking legs. Scale bar = 10  $\mu$ .

Figure II.8 Scanning electron micrograph showing the cuticle in the coxal groove. Scale bar = 10  $\mu$ .

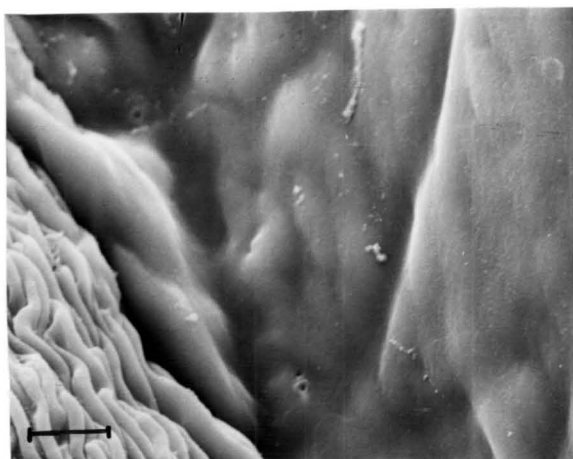
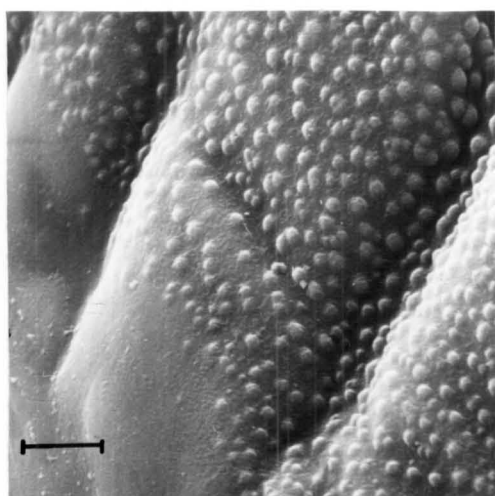
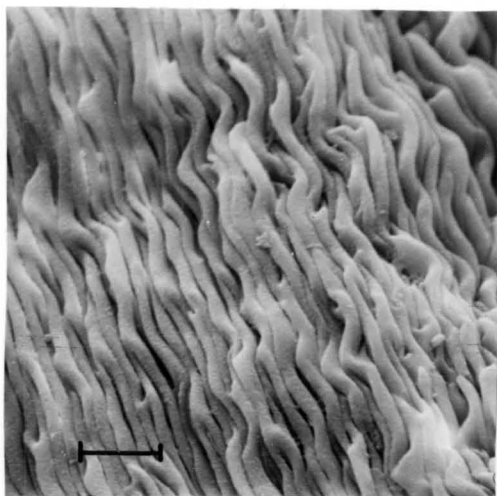
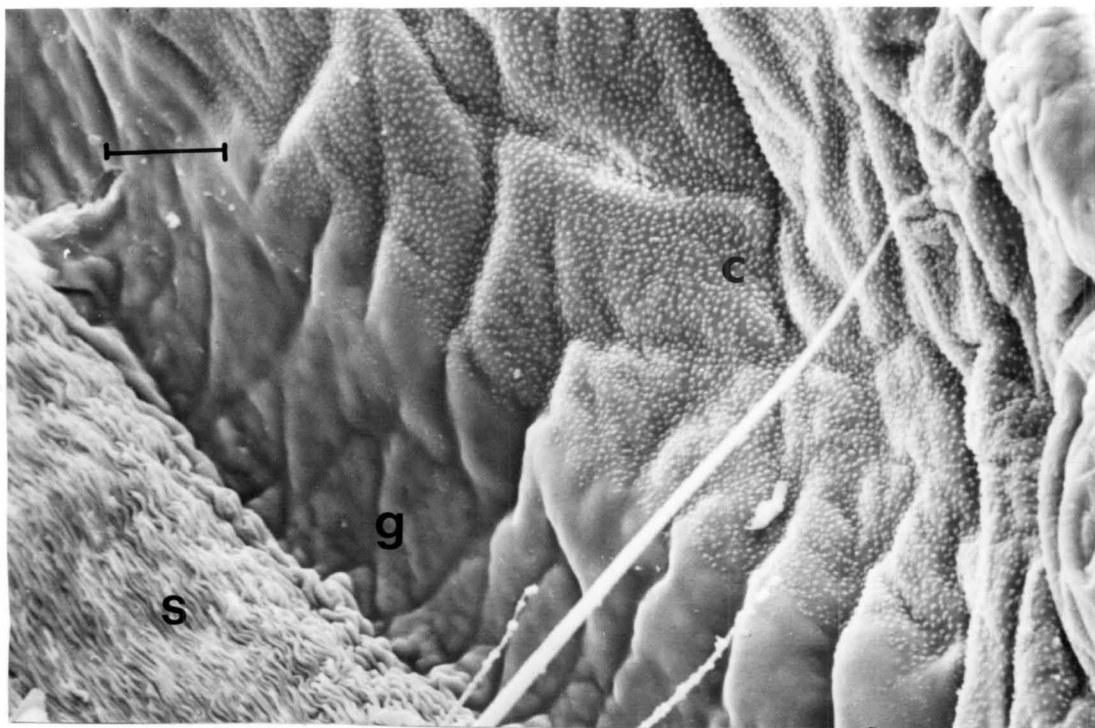




Table II.2 The composition of coxal fluid secreted by hydrated spiders fed continuously for 40 minutes on the abdominal contents of freshly killed cockroach nymphs, and the composition of the haemolymph before and after feeding. All values Mean  $\pm$  S.E.M.

	Coxal fluid	Haemolymph	
		Before	After
Rate (n = 12) $\mu\text{l hr}^{-1}\text{coxal opening}^{-1}\text{gm}^{-1}$	$18.2 \pm 1.8$	—	—
Osmotic pressure (n = 12) $\text{mOsm (KgH}_2\text{O)}^{-1}$	$309 \pm 38.6^{***}$	$460 \pm 14.7$	$468 \pm 12.2$
Na $\text{mM l}^{-1}$ (n = 12)	$125.4 \pm 13.1^{***}$	$233 \pm 7.6$	$239 \pm 6.8$
K $\text{mM l}^{-1}$ (n = 12)	$35.6 \pm 2.9^{***}$	$7.6 \pm 0.5$	$5.46 \pm 0.26^{++}$
Na : K ratio (n = 12)	$3.8 \pm 0.53^{***}$	$31.3 \pm 2.3$	$44.4 \pm 2.5^{++}$
Inulin ratio (n = 8)	$1.29 \pm 0.05$	—	—

\* Indicates that values of coxal fluid significantly different from values of haemolymph when compared by paired Student t-test.

† Indicates haemolymph values after feeding significantly different from values before feeding when compared by paired Student t-test.

Levels of significance: \* or †,  $0.05 > p > 0.01$ ; \*\* or ††,  $0.01 > p > 0.001$ ; \*\*\* or †††,  $0.001 > p$ .

The coxal fluid/haemolymph inulin ratio was estimated by comparing the ( $^3\text{H}$ )-inulin activity of the pooled coxal fluid sample to the mean of the haemolymph activity before and after feeding. Attempts to test the ability of the coxal glands to absorb inulin were thwarted by the inaccessibility of the coxal openings and the closure of the coxal opening, when the gland was not secreting, preventing the back injection of inulin into the gland. The measured inulin clearance ratio of the coxal gland,  $1.29 \pm 0.05$  ( $\bar{X} \pm \text{S.E.M.}$ , n = 8), was only slightly, although significantly (paired Student t test,  $0.001 > p$ ), greater than one.

The concentrations of Na ( $125 \text{ mM l}^{-1}$ ) and K ( $35.6 \text{ mM l}^{-1}$ ) were approximately one half and five times, respectively, the concentrations measured in the haemolymph. Chloride measurements were not complete, however, in three coxal fluid samples in which it was measured Cl was the major anion present (Na,  $139.3 \text{ mM l}^{-1}$ ; K,  $30.2 \text{ mM l}^{-1}$ ; Cl,  $127.3 \text{ mM l}^{-1}$ ). In hydrated spiders the coxal fluid was at all times hypo-osmotic to the haemolymph and the major cations and anions, Na, K and Cl, were the major osmolar effectors accounting for 90% of the osmotic pressure of the coxal fluid.

#### II.3.2.b Changes in the composition of the haemolymph with feeding

Collection of haemolymph samples during feeding invariably resulted in the interruption of feeding, struggling by the spider and an end to coxal excretion. Thus it was not possible to monitor changes in the haemolymph composition during feeding and coxal excretion. However, comparison of the haemolymph samples collected immediately before and after feeding give some indication of the combined effect of feeding and coxal excretion on the haemolymph composition.

Shown in Table II.2 is the composition of the haemolymph immediately before and after feeding spiders for 40 minutes. These spiders were taken from a culture that was provided with food once a week. The composition of the haemolymph before feeding is in good agreement with what was observed previously for fed spiders (Section I). Following feeding there were small increases in the osmotic pressure and Na concentration of the haemolymph, but, notably the K concentration decreased from  $7.6 \text{ mM l}^{-1}$  before feeding to  $5.46 \text{ mM l}^{-1}$  after feeding.

#### II.3.3 Changes in Coxal Fluid Composition During Feeding

Coxal fluid was collected for a series of set intervals throughout a forty minute feeding period and the rate of excretion and composition of the coxal fluid determined for each interval. The results are summarised in Figure II.9.

During feeding there was a 47% reduction in the rate of coxal excretion. Most of the reduction occurred within the first ten minutes of feeding when the rate fell from an initial value of  $0.45 \text{ } \mu\text{l min}^{-1}\text{coxal opening}^{-1}\text{gm}^{-1}$  to  $0.3 \text{ } \mu\text{l min}^{-1}\text{coxal opening}^{-1}\text{gm}^{-1}$ . Thereafter the rate

Figure II.9      Variation in the composition of the coxal fluid with feeding.    Vertical bars =  $\pm 1$  S.E.M.,  $n = 8$ .

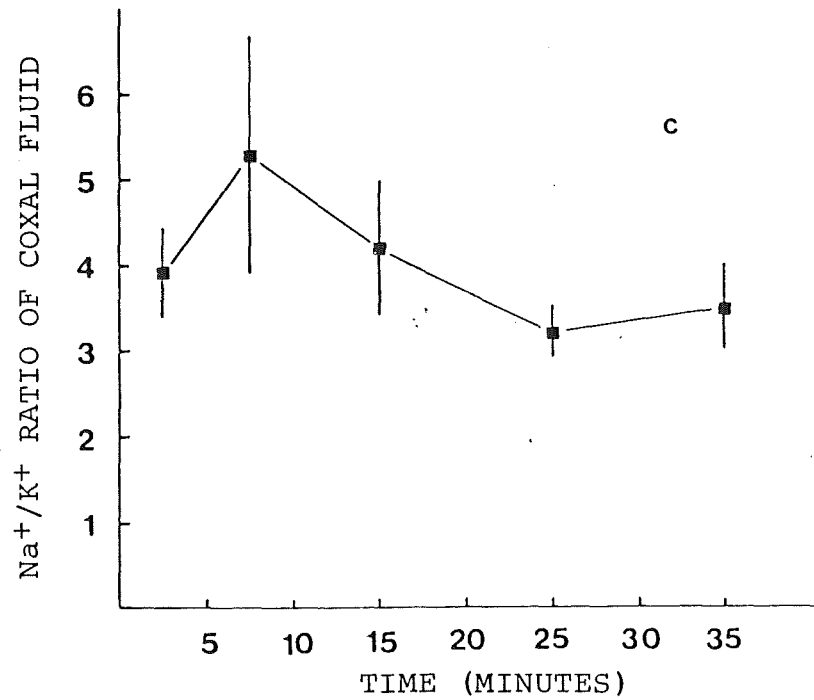
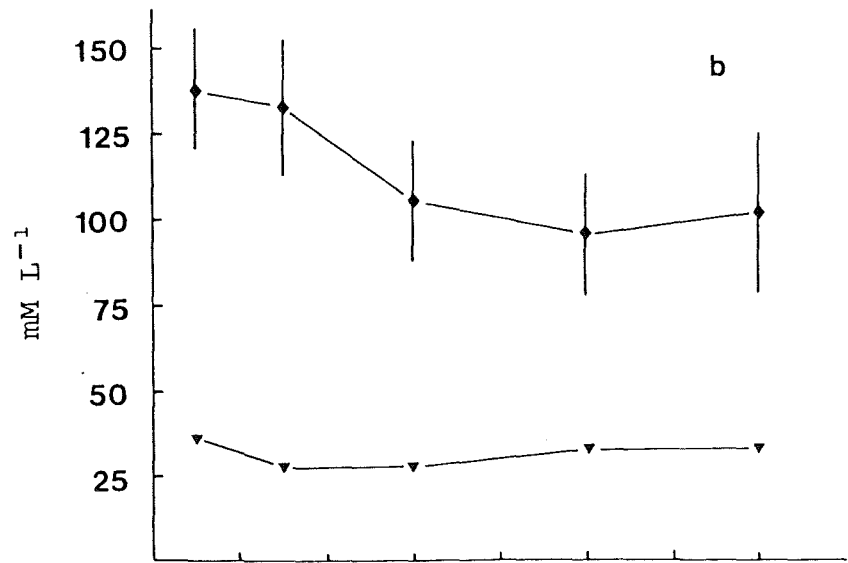
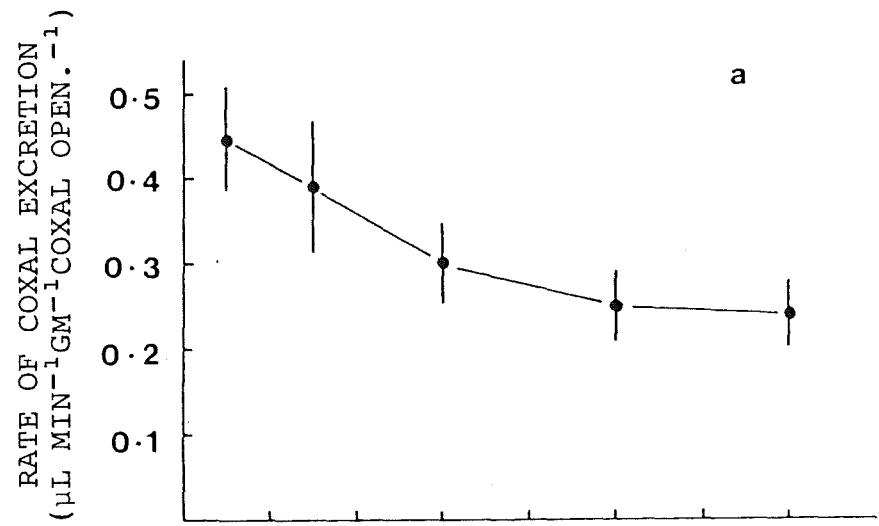
(a)    Rate of excretion.

(b)    Cation concentration

◆ —◆ Sodium

▼ —▼ Potassium

(c)    Na/K ratio.



was reasonably constant, although it did continue to decline slightly.

It is difficult to determine whether the decline in rate was real or simply an artifact resulting from the way in which the spiders were restrained and fed during the collection of coxal fluid. All spiders fed continuously for a period in excess of forty minutes without manual stimulation of the chelicerae after the onset of feeding. As mentioned previously, unrestrained spiders do not feed continuously. Therefore, the initial rate of excretion may be more representative of the true rate of excretion.

The coxal fluid had an initial Na concentration of  $137.5 \text{ mM l}^{-1}$ , however, after ten minutes of feeding this had fallen to  $105 \text{ mM l}^{-1}$  and then showed little variation for the remainder of the feeding period. Changes in K concentration did not exhibit any consistent trend. There was an initial decrease in concentration in the first five minutes of feeding, but it gradually increased and had returned to a concentration close to the initial value at the completion of the meal.

Of particular significance is the Na/K ratio of the coxal fluid. Pooled samples of coxal fluid had a Na/K ratio of 3.8 (Table II.2), and throughout the forty minute feeding interval the ratio was never lower than 3.0 and was, at times, as high as 5.3 (Fig. II.9.c). It was shown in Section I that  $4.8 \text{ } \mu\text{M}$  of Na and  $1.3 \text{ } \mu\text{M}$  of K were lost in the food debris left after the completion of a meal by *P. antipodiana*. This is a ratio of 3.7 and should be representative of the fluid excreted into the food during feeding.

#### II.4 RESULTS - DIETARY SALT LOADING

*P. antipodiana* would readily feed on cockroach nymphs which had been injected with NaCl or KCl solutions. The willingness of the spiders to feed on such prey allowed them to be subject to the physiological stress of dietary salt loading. This was carried out in an attempt to obtain some insight into the operation of the two potential excretory systems. There is evidence in the normal hydrated spider that the coxal excretion may be incidental to the feeding process (Section I), the low rate of excretion of Na via the anus resulting from a low net ingestion of Na during feeding because of coxal loss in the food debris. Alternatively,

the mechanism(s) by which anal excreta are produced may be incapable of Na excretion. If so, coxal excretion would be of considerable importance to the animal.

Spiders were subject to dietary Na and K salt loading by injecting cockroach nymphs with 2 molar solutions of NaCl and KCl. Nymphs of approximately 0.15 gm were selected and injected with sufficient NaCl or KCl to raise their Na or K content to 40  $\mu\text{M animal}^{-1}$ . The approximate concentration of salts in the injected nymphs is shown in Table II.3. Control animals were provided with similar sized meals of normal composition.

Table II.3 The composition of cockroach nymphs provided to *P. antipodiana* under different dietary regimes. Na and K, mM (gm dry weight) $^{-1}$ ; water, ml (gm dry weight) $^{-1}$ .

	Normal cockroaches	Na loaded cockroaches	K loaded cockroaches
Na	0.162	1.06	0.15
K	0.319	0.273	1.06
Na/K	0.50	3.8	0.14
H <sub>2</sub> O	3.0	3.5	3.25

#### II.4.1 The Effect of Dietary Salt Loading on the Composition of the Haemolymph, Coxal Fluid and Stercoral Fluid

The coxal fluid and stercoral fluid of spiders fed on salt loaded prey were obtained from two different groups. For the collection of coxal fluid, spiders were provided with salt loaded prey for twelve hours and then those that had successfully captured and consumed prey were used for coxal fluid collection. Stercoral fluid was collected from a second group of spiders which had fed on salt loaded prey 24 hours earlier. Haemolymph samples were collected from all spiders. Control values were obtained from Section I and above.

The effects of dietary salt loading on the stercoral fluid composition are listed in Table II.4a and coxal fluid composition in Table II.4b.

*Via via*

Table II.4a The ionic composition of the haemolymph and coxal fluid of spiders fed salt loaded prey. Normal food, live cockroach nymphs directly from culture. Na loaded food, 0.15 gm cockroach nymphs injected with 40  $\mu$ M of NaCl. K loaded food, similar sized nymphs injected with 40  $\mu$ M of KCl. Haemolymph values mean of samples before and after feeding. All values  $X \pm S.E.M.$

Food Type		Rate ( $\mu$ l hr <sup>-1</sup> gm <sup>-1</sup> cg <sup>-1</sup> )	Osmotic Press. (mOsm KgH <sub>2</sub> O <sup>-1</sup> )	Na (mM l <sup>-1</sup> )	K (mM l <sup>-1</sup> )	Inulin Ratio Coxal Fluid/Haem.
Normal (n = 12)	Coxal	18.2 $\pm$ 1.8	298 $\pm$ 26	125.4 $\pm$ 13.1	35.6 $\pm$ 2.9	1.29 $\pm$ 0.05
	Haemolymph	-	468 $\pm$ 12.2	234 $\pm$ 6.8	7.1 $\pm$ 1.23	
Na loaded (n = 6)	Coxal	22.6 $\pm$ 3.2	592.2 $\pm$ 52*	264 $\pm$ 29.6***	21.4 $\pm$ 1.1***	1.23 $\pm$ 0.6
	Haemolymph	-	635 $\pm$ 11.0***	294.8 $\pm$ 9.1***	8.6 $\pm$ 1.15	
K loaded (n = 5)	Coxal	15.2 $\pm$ 1.8	287 $\pm$ 27.8	123.9 $\pm$ 64.0	33.1 $\pm$ 1.3	1.30 $\pm$ 0.08
	Haemolymph	-	443.5 $\pm$ 13.6	237 $\pm$ 10.2	6.5 $\pm$ 0.66	

\* Indicates means of animals fed modified diets significantly different from corresponding control values (animals fed normal prey) by unpaired Student t test. Level of significance - \*, 0.05 > p > 0.01; \*\*, 0.01 > p > 0.001; \*\*\*, 0.001 > p.

Table II.4b The composition of the haemolymph and stercoral fluid of spiders fed salt loaded prey 24 hours prior to sampling. Normal, Na and K loaded food as in Table II.4a. All values  $\bar{X} \pm \text{S.E.M.}$

Food Type		Osmotic Press. (mOsm $\text{KgH}_2\text{O}^{-1}$ )	Na (mM $\text{l}^{-1}$ )	K (mM $\text{l}^{-1}$ )	Na/K ratio
Normal (n = 9)	Haemolymph	$515 \pm 10.4$	$226 \pm 5.6$	$4.5 \pm 0.3$	$0.28 \pm 0.07$
	Stercoral fluid	$504 \pm 10.2$	$34.6 \pm 8.6$	$125.4 \pm 6.4$	
Na loaded (n = 8)	Haemolymph	-	$293 \pm 16.0^{***}$	$6.6 \pm 0.3^{***}$	$3.56 \pm 0.4^{***}$
	Stercoral fluid	-	$253 \pm 21.6^{***}$	$73.6 \pm 4.8^{***}$	
K loaded (n = 9)	Haemolymph	-	$216 \pm 7.3$	$4.2 \pm 0.2$	$0.002 \pm 0.10^*$
	Stercoral fluid	-	$23.2 \pm 12.5$	$227.3 \pm 23.4^{***}$	

\* Indicates means of animals fed modified diets significantly different from corresponding control values (animals fed normal prey) by unpaired Student t test. Level of significance - \*,  $0.05 > p > 0.01$ ; \*\*,  $0.01 > p > 0.001$ ; \*\*\*,  $0.001 > p$ .



In spite of a three-fold increase in the K concentration of the food (Table II.3) the haemolymph K concentration decreased slightly with feeding in both groups. There was a marked increase in the K concentration of the stercoral fluid from 125.4 to 227.3 mM l<sup>-1</sup> but no real change in the K concentration of coxal fluid. In contrast, Na loading resulted in a 60-70 mM l<sup>-1</sup> increase in haemolymph Na concentration and increases in both the stercoral fluid and coxal fluid Na concentrations. The stercoral fluid Na concentration increased from 34.6 mM l<sup>-1</sup> in control spiders to 253 mM l<sup>-1</sup> in Na loaded spiders. Interestingly, there was also a reduction in the K concentration of the stercoral fluid. The coxal fluid increased in Na concentration from 125.4 to 264 mM l<sup>-1</sup> and, similarly, there was a decrease in the K concentration. The increase in the Na concentration of the coxal fluid was a result of reduced reabsorption of Na rather than reabsorption of water as there was little difference between the haemolymph/coxal fluid inulin ratios of both groups.

#### II.4.2 The Effect of Dietary Salt Loading on Excretion Via the Anus and the Coxal Glands

Three groups of spiders of approximately equal size were used. One group serving as a control was fed normal prey, the other two groups were fed either Na loaded or K loaded prey.

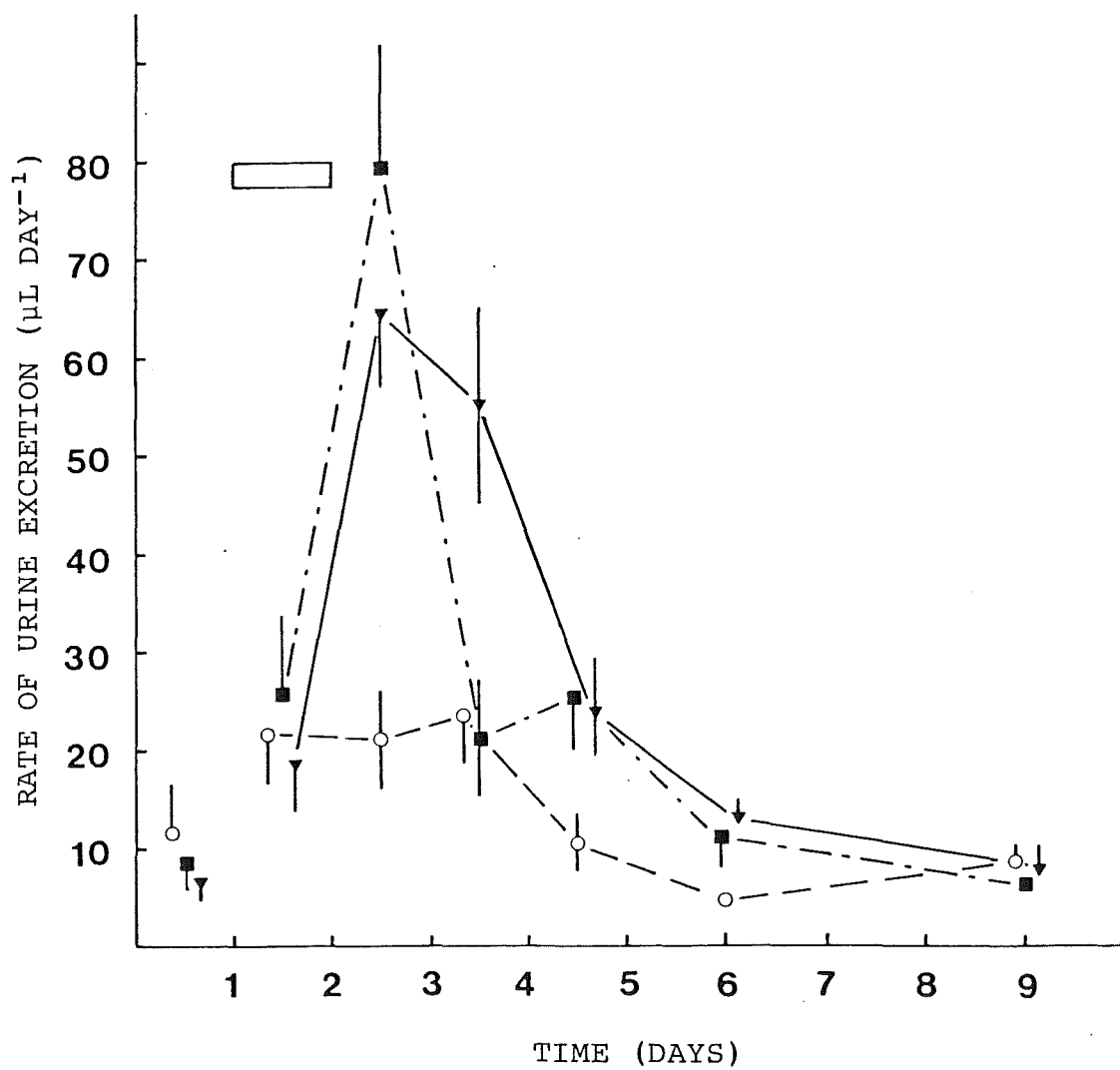
Spiders were injected with <sup>22</sup>Na to aid the location of excreted coxal fluid. After 48 hours equilibration following injection, normal rates of anal and coxal excretion were measured and then the spiders were provided with food. Each spider was provided with three cockroach nymphs for 24 hours, and the rates of urine production and Na and K excretion via the anus were measured in the normal manner during feeding and for eight days after the completion of the meal. At all times the spiders had access to water.

The injection of such large quantities of KCl killed the cockroach nymphs, but the spiders often readily fed spontaneously on the freshly dead nymphs, and at other times were easily encouraged to feed by brushing the dead nymphs against the chelicerae and cephalothorax of the spiders.

The information from this single experiment is presented in two sections, one concerned with anal excretion and the other coxal excretion.

Figure II.10      Effect of dietary salt loading on the rate of urine excretion by *P. antipodiana*.      Horizontal bar represents feeding period.      Vertical bar =  $\pm 1$  S.E.M.

- — — ○ Control spiders fed normal food, (n = 8).
- ·····■ Spiders fed K loaded prey, (n = 10).
- ▼ ———▼ Spiders fed Na loaded prey, (n = 8).



A summary is presented at the end of the second section.

#### II.4.2.a The effects of dietary salt loading on anal excretion

The rates of urine excretion of spiders fed meals with excess Na or K added and control spiders fed normal food are shown in Figure II.10. Prior to feeding, urine excretion by all three groups was similar to that reported for starved spiders in Section I, varying between 6 and 12  $\mu\text{l day}^{-1}$ . The response of the control spiders to feeding was very similar to that reported for spiders fed a single meal in Section I. Urine excretion increased to a rate of 21.5  $\mu\text{l day}^{-1}$  during feeding and was maintained at this elevated level for three days before returning to prefeeding levels.

In both Na and K loaded spiders, during feeding the rate of urine excretion increased to a level similar to that of control spiders. However, it continued to increase after the completion of the meal rising to a peak of 79.5  $\mu\text{l day}^{-1}$  and 65  $\mu\text{l day}^{-1}$  for K and Na loaded spiders, respectively, in the day following feeding. The following day, the rate of excretion of the K loaded spiders dropped to 21  $\mu\text{l day}^{-1}$ , a rate equivalent to that of the control spiders for the same interval, but while in the control spiders the rate fell to prefeeding levels the next day, in K loaded spiders urine excretion was maintained at an elevated rate for a further day before returning to prefeeding levels. Following the peak of urine excretion observed during feeding by Na loaded spiders, the rate only fell slightly in the next day then decreased gradually to prefeeding and control values over the next three days.

Control spiders responded to feeding with an increased K excretion for three days (Figure II.11) and a slight increase in Na excretion during the feeding period (Figure II.12), a similar result to that observed in Section I.

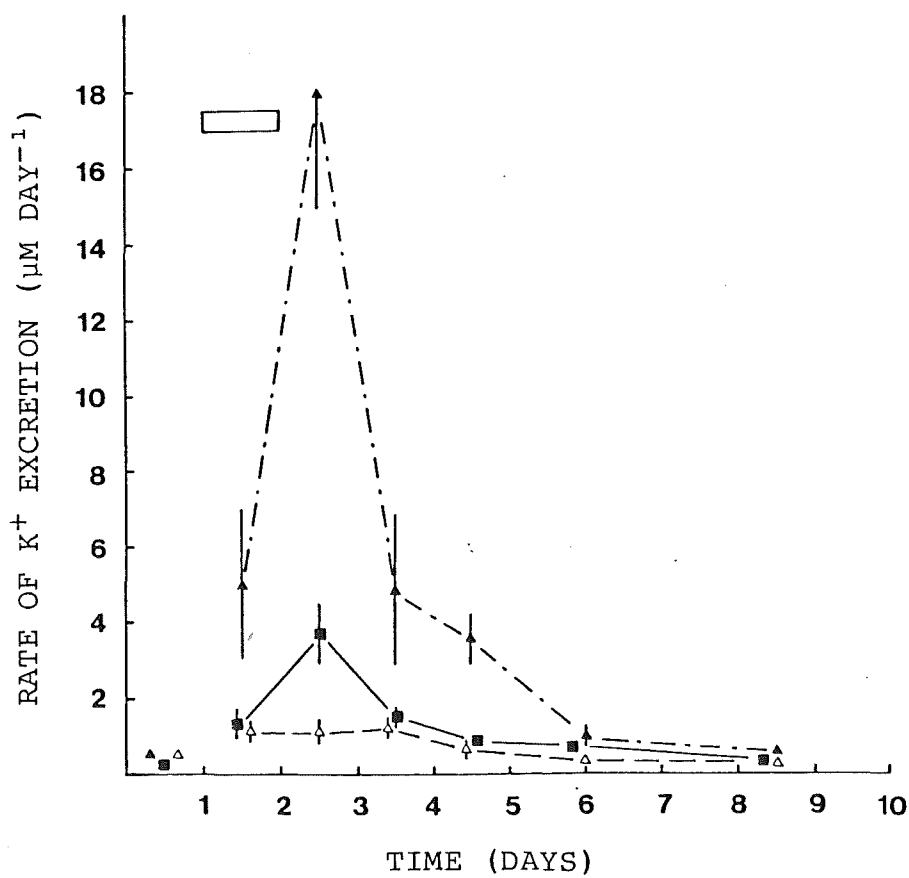
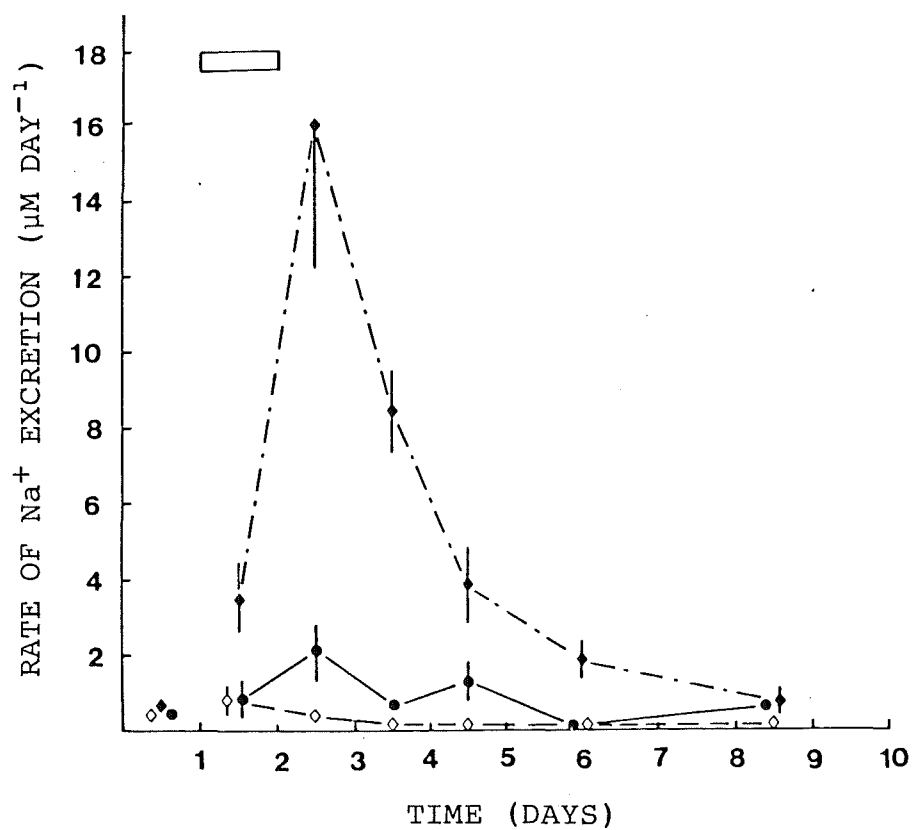
Increased dietary salt intake resulted in marked increases in the rate of both Na and K excretion in the excreta. Sodium excretion by Na loaded spiders increased from prefeeding levels of 0.5  $\mu\text{M day}^{-1}$  to 3.4  $\mu\text{M day}^{-1}$  during feeding and 16.0  $\mu\text{M day}^{-1}$  the day following feeding. There was a subsequent decline in the rate of Na excretion, however, it did not return to prefeeding levels until 120 hours after feeding (Figure II.11).

Figure II.11 Effect of dietary salt loading on the excretion of Na in the excreta of *P. antipodiana*. Horizontal bar represents period of feeding. Vertical bar =  $\pm 1$  S.E.M.

- ◇ — — ◇ Control spiders fed normal prey, (n = 8).
- ◆ — — ◆ Spiders fed Na loaded prey, (n = 10).
- — — ● Spiders fed K loaded prey, (n = 8).

Figure II.12 Effect of dietary salt loading on the excretion of K in the excreta of *P. antipodiana*. Horizontal bar represents period of feeding. Vertical bar =  $\pm 1$  S.E.M.

- △ — — △ Control spiders fed normal prey, (n = 8).
- ▲ — — ▲ Spiders fed K loaded prey, (n = 8).
- — — ■ Spiders fed Na loaded prey, (n = 10).



Interestingly, the rate of K excretion of Na loaded spiders also increased (Figure II.12). During the feeding period, K excretion by Na loaded spiders was similar to that observed in control spiders, however, during the day following feeding, K excretion was significantly greater than control values (Figure II.12). The quantity of K ingested by the spiders fed Na loaded food ( $25.5 \mu\text{M K}$ ) was greater than that ingested by the control animals ( $17.2 \mu\text{M K}$ ), therefore an increased K excretion by these animals would be predicted if the K balance was to be maintained.

Not unexpectedly, dietary K loading resulted in a large increase in the rate of anal K excretion (Figure II.12). Maximum K excretion ( $18.0 \mu\text{M day}^{-1}$ ) was achieved the day following feeding, and during the following four days anal K excretion declined to prefeeding and control levels. In these K loaded animals the rate of anal Na excretion was, at times, higher than that observed in the control animals (Figure II.11). Control spiders and K loaded spiders ingested similar quantities of Na during feeding, therefore, increased Na excretion by the K loaded spiders would not be expected. The increased Na excretion of the K loaded spiders occurred during peak urine production and probably reflects a delay in the response of the excretory system in compensating for the increased urinary excretion. Notably, during the following day urine excretion by the K loaded animals was again high, but Na excretion was no longer greater than that observed in the control animals.

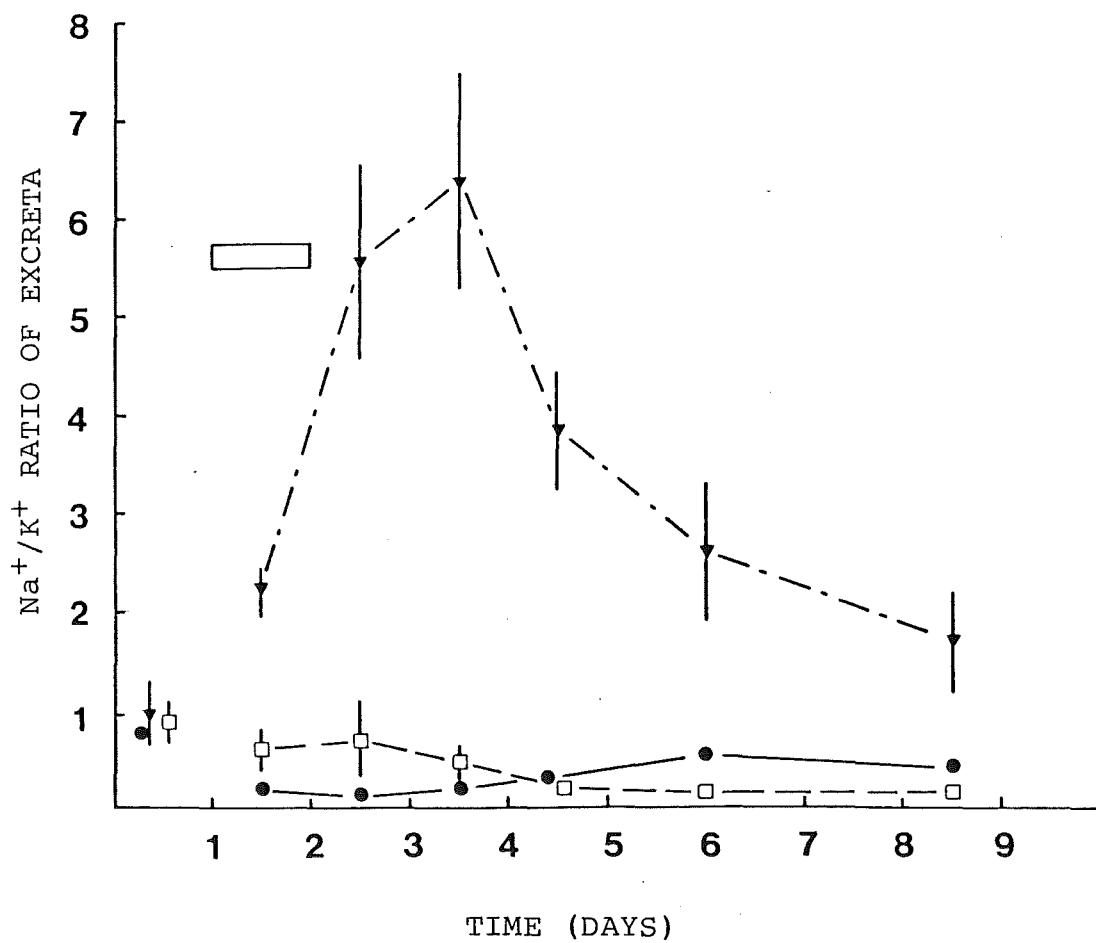
It should be noted that these increases in Na excretion by K loaded animals and K excretion by Na loaded animals were small compared with increases in rates of Na or K excretion in response to an increased Na or K load in the meal.

The Na/K ratio of the excreta of spiders fed different food is shown in Figure II.13. Prior to feeding, the values recorded for all three groups corresponded to values previously observed for starved spiders (Section I). The Na/K ratio of the excreta of control animals decreased following feeding, although it was two days after the completion of the meal before the ratio fell to levels previously observed in feeding spiders (Section I). It is interesting to note that the Na/K ratio of these control animals had not returned to prefeeding levels eight days after the completion of the meal. Predictably, in response to increased Na ingestion there was a marked increase in the Na/K ratio of the

Figure II.13      Effect of dietary salt loading on the Na/K ratio of the excreta of *P. antipodiana*.    Horizontal bar represents the feeding period.    Vertical bar =  $\pm 1$  S.E.M.

- — — □ Control spiders fed normal prey, (n = 8).
- ▼ — — — ▼ Spiders fed Na loaded prey, (n = 10).
- — — — ● Spiders fed K loaded prey, (n = 8).





excreta, from 0.95 before feeding to 6.4 one day after the completion of the meal, and even eight days after the completion of the meal the Na/K ratio of the excreta of Na loaded animals had not returned to control levels. Increased K content of the diet resulted in an immediate decrease in the Na/K ratio of the excreta, from 0.75 to 0.25, and a further decrease the following day. The ratio subsequently increased and had returned to prefeeding levels four days after the completion of the meal.

#### II.4.2.b The effect of dietary salt loading on coxal excretion

As well as excretion via the anus (II.4.2.a above), excretion by the coxal glands was monitored in spiders fed salt loaded prey.

The excretion of Na and K in the coxal fluid of control spiders and spiders fed Na or K loaded prey is shown in Figure II.13. Sodium loss, through coxal excretion, in the prey debris was estimated from the  $^{22}\text{Na}$  content of the prey debris and the mean of the  $^{22}\text{Na}$  specific activity of the coxal fluid before and after feeding. Small samples of coxal fluid were collected from the posterior coxal openings of the spiders before feeding and the specific activity of these determined. These were compared to the specific activity of the coxal fluid dried on the filter paper lining the experimental chambers following feeding.

$$\text{e.g. } \mu\text{M Na lost in debris} = \frac{\text{MBq} \times ^{22}\text{Na in debris}}{\text{Mean coxal fluid } ^{22}\text{Na specific activity}}$$

It should be noted that this is not equivalent to the total Na excretion by the coxal glands as significant amounts will be re-ingested as feeding proceeds. The amount of K lost in the prey debris by these animals was estimated in the same manner as in Section I.

In the absence of a convenient K isotope comparable to  $^{22}\text{Na}$  it was not possible to obtain a direct estimate of the amount of K lost in the debris following feeding by spiders on K loaded prey. However, feeding experiments in which spiders were fed several K rich prey indicated that there was little change in the composition of the coxal fluid with dietary K loading (Section II.4.1). Therefore, the amount of Na excreted by the coxal glands of K loaded spiders was calculated as for Na loaded spiders, and then the K excretion calculated from the

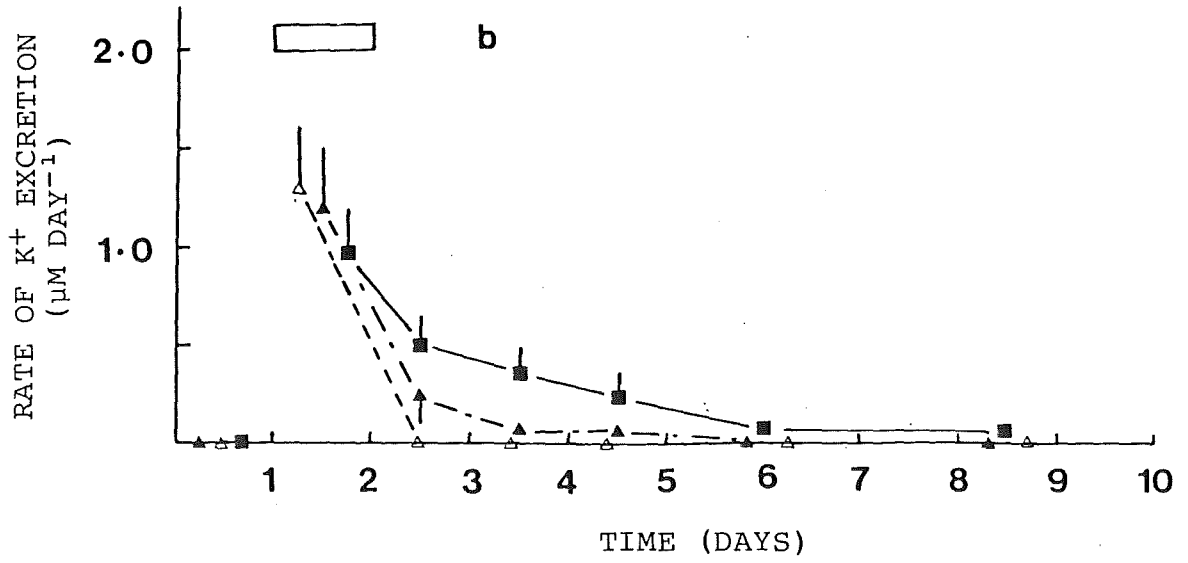
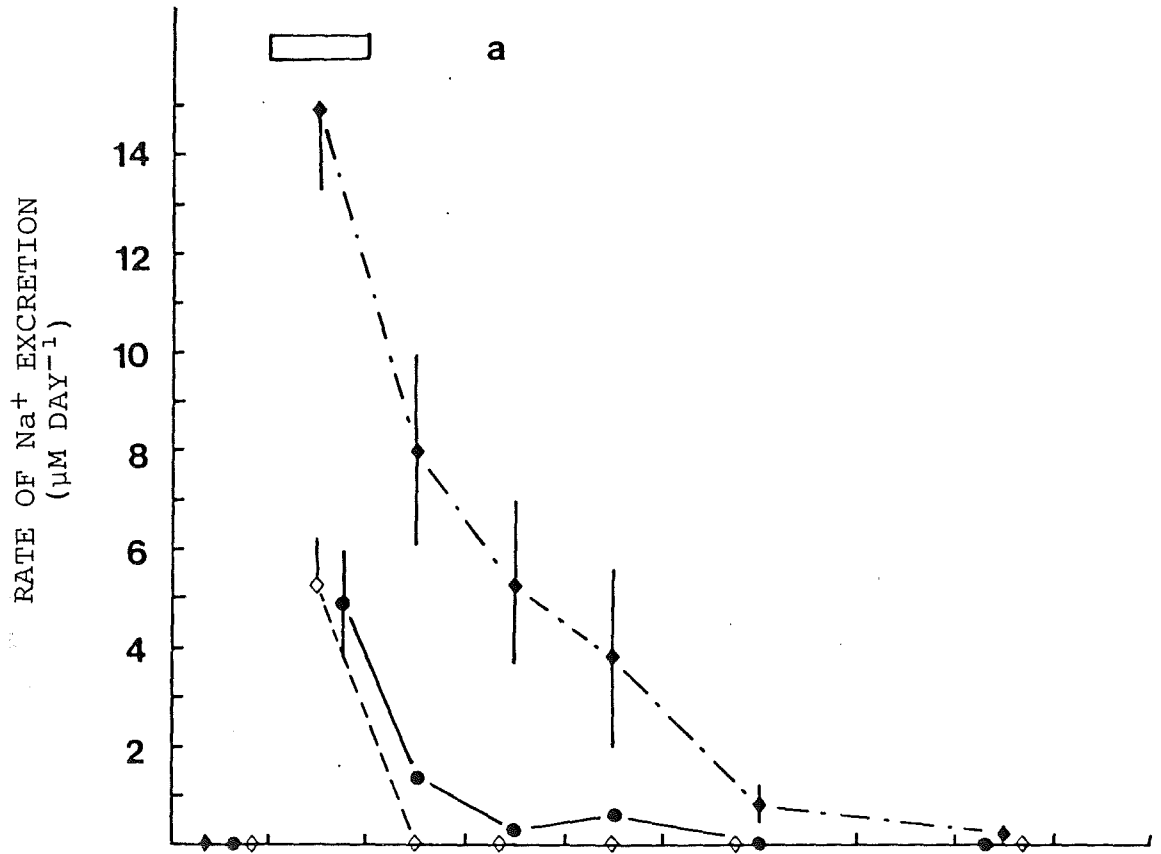
Figure II.14 Effect of dietary salt loading on excretion by the coxal glands of *P. antipodiana*. Horizontal bar represents feeding period. Vertical bar =  $\pm 1$  S.E.M.

(a) The excretion of Na

- ◇ --- ◇ Control spiders fed normal prey, (n = 8).
- ◆ ---- ◆ Spiders fed Na loaded prey, (n = 10).
- ——— ● Spiders fed K loaded prey, (n = 8).

(b) The excretion of K

- △ --- △ Control spiders fed normal prey, (n = 8).
- ▲ ---- ▲ Spiders fed K loaded prey, (n = 8).
- ——— ■ Spiders fed Na loaded prey, (n = 10).



Na/K ratio of the coxal fluid collected from spiders fed K loaded prey.

$$\begin{array}{lcl} \text{e.g. } \mu\text{M K lost from spiders into} & & \text{Na/K ratio of coxal} \\ \text{the prey debris through} & = & \text{fluid collected from} \\ \text{coxal excretion} & & \text{spiders feeding on} \\ & & \text{K loaded food.} \end{array}$$

Coxal fluid that had dried on the filter paper lining the experimental chambers was difficult to locate with either ultra violet or normal light. Therefore, coxal fluid excreted after the completion of the meal was located by drying the filter paper, subdividing it into a number of small pieces and measuring the  $^{22}\text{Na}$  activity of the individual pieces. Regions of high  $^{22}\text{Na}$  activity were transferred to measured volumes of 1%  $\text{HNO}_3$  and the Na and K content determined.

In contrast to normally feeding spiders, those fed Na loaded prey were observed with drops of fluid of varying size suspended from the pre-oral region after the completion of the meal. This fluid resembled coxal fluid, rather than midgut fluid, in that it was transparent and non-viscous in appearance. At times these drops were seen to touch and be absorbed onto the filter paper lining the cages, and there was considerable evidence for the loss of Na from the Na loaded spiders through coxal excretion after the completion of the meal (Figure II.14). It is not clear whether such coxal excretion could occur under normal circumstances or whether it is essentially accidental. The web tube in which *P. antipodiana* is normally found is hydrophobic, thus fluid drops such as this would have to be actively dislodged under normal conditions. Disturbance of such spiders always resulted in rapid ingestion of the fluid, unless it contacted the filter paper.

In spite of the reservations concerning the excretory nature of the coxal fluid lost on the filter paper following feeding, there was considerable evidence for an excretory role of the coxal fluid actually lost into the Na loaded prey (Figure II.14). Spiders fed Na loaded prey lost greater amounts of Na ( $14.9 \mu\text{M}$ ) in the prey debris than controls ( $5.2 \mu\text{M}$ ), or spiders fed K loaded prey ( $4.9 \mu\text{M}$ ). As noted above, there was a continued loss of coxal fluid from the Na loaded spiders following feeding. This continued for a period of up to eight days after the completion of the meal, and was responsible for the elimination of  $19.6 \mu\text{M}$  or 27% of the total Na excreted (Table II.5). There was no evidence of increased K loss in the prey debris of spiders fed K loaded prey. However,

Table II.5 Summary of anal and coxal excretion of Na and K by spiders fed prey of different cation composition. Controls fed cockroach nymphs from culture. Na loaded food, 0.15 gm cockroach nymphs injected to raise Na <sup>content</sup> to 40  $\mu$ M. K loaded food, similar size cockroach nymphs injected with sufficient K to raise total K content to 40  $\mu$ M.

		Total Ingested	Coxal Excretion			Anal Excretion	Total Excretion
			During feeding	After feeding	Total		
Control (n = 8)	Na $\mu$ M	8.3	5.2	Trace	5.2	2.6	7.8
	K $\mu$ M	17.2	1.4	Trace	1.4	7.9	9.3
Na loaded food (n = 10)	Na $\mu$ M	71.8	14.9	19.6	34.5	37.3	71.9
	K $\mu$ M	25.5	0.96	1.2	2.3	10.2	12.3
K loaded food (n = 8)	Na $\mu$ M	8.7	4.9	2.1	7.0	3.6	10.6
	K $\mu$ M	50.2	1.2	0.3	1.5	38	39.5

the determination of this was dependent upon measurements of the Na/K ratio of the coxal fluid excreted by spiders fed K loaded food rather than direct measurements of the amount of K, for example, in the form of  $^{42}\text{K}$ , lost from the spider into the debris. Measurements of this kind are required to confirm the indirectly determined estimates presented here. The K loaded spiders did continue to excrete small amounts of coxal fluid for three days following the completion of the meal (Figure II.14). The quantities of K eliminated in this fluid were very small, however, compared to the total K eliminated by the anal system.

A summary of Na and K excretion in the coxal fluid and the excreta of spiders fed food of different cation composition is shown in Table II.5. In control spiders, although limited to periods of feeding, the coxal glands were the major route for Na excretion, eliminating 66% of the total excreted Na. Conversely, the anal system was responsible for most of the K excreted, eliminating 85% of the total excreted K. Similar divisions of Na and K excretion were observed in spiders fed normal prey in Section I. In spiders fed K loaded prey there was a marked increase in total K excretion. This was due almost entirely to increased anal excretion (controls, 7.9  $\mu\text{M}$  K excreted via anus; K loaded, 37.3  $\mu\text{M}$  K excreted via the anus), the coxal glands of these spiders excreting similar quantities as the controls. The coxal glands of the K loaded spiders did, however, retain the role of the major route for Na excretion, eliminating 3.6  $\mu\text{M}$  Na or 66% of the total Na excreted. Significantly, in the Na loaded spiders the increase in total Na excretion resulted from increased coxal excretion as well as increased anal excretion. The coxal glands excreted a total of 34.5  $\mu\text{M}$  of Na during and after feeding, and although this represents only 48% of the total Na excreted, in absolute terms it is a large increase compared to the controls. The increase in Na excretion by the anal system was also large, from 2.6  $\mu\text{M}$  in the controls to 37.3  $\mu\text{M}$  Na, and demonstrates the ability of the anal system to excrete significant quantities of Na, a feature which is not obvious in the control animals.

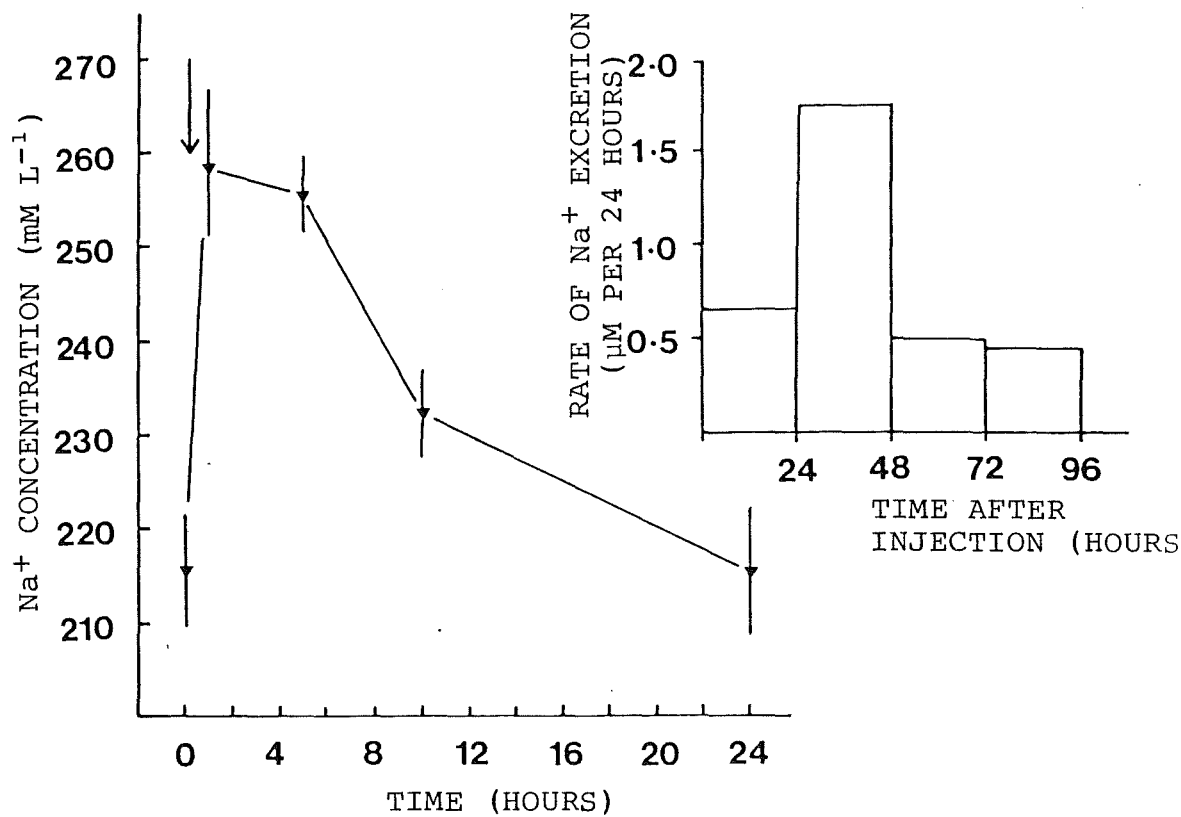
## II.5 RESULTS - SALT LOADING BY INJECTION

Salt loading was also achieved by injection into the haemolymph. This eliminated any contribution of the gut which may act as a homeostatic mechanism limiting the assimilation of ions into the haemolymph. Unfortunately, although *P. antipodiana* would tolerate large injected loads

Figure II.15    Change in the Na concentration of the haemolymph following injection with 30  $\mu$ M NaCl per gm spider. Arrow indicates time of injection. Vertical bars =  $\pm$  1 S.E.M. (n = 5).

Inset    The rate of Na excretion in the excreta of *P. antipodiana* following injection of 30  $\mu$ M NaCl per gm spider into the haemolymph.





of Na, the injection of KCl ( $30 \mu\text{M gm}^{-1}$ ) invariably resulted in the death of the animals. Therefore it was not possible to manipulate haemolymph K in this manner.

The ability of *P. antipodiana* to regulate the haemolymph Na concentration was tested by injecting spiders with  $30 \mu\text{M NaCl}$  per gm and monitoring changes in the haemolymph Na concentration. The rate of Na excretion in the excreta was also measured.

As a result of injection, the haemolymph Na concentration increased from  $215$  to  $259 \text{ mM l}^{-1}$ , however, it returned to pre-injection levels within 24 hours (Figure II.15). The observed increase was less than that predicted from measurements of the Na space of *P. antipodiana* ( $42.3 \pm 2.3\%$  of bodyweight;  $\bar{X} \pm \text{S.E.M.}$ ,  $n = 5$ ), and the known amount of Na injected. Assuming rapid and equal mixing of the injected Na throughout the Na space, an increase in haemolymph Na concentration of  $70 \text{ mM l}^{-1}$  would have been expected. Thus, significant quantities of Na were removed from the haemolymph within an hour, the time between injection and collection of the first sample.

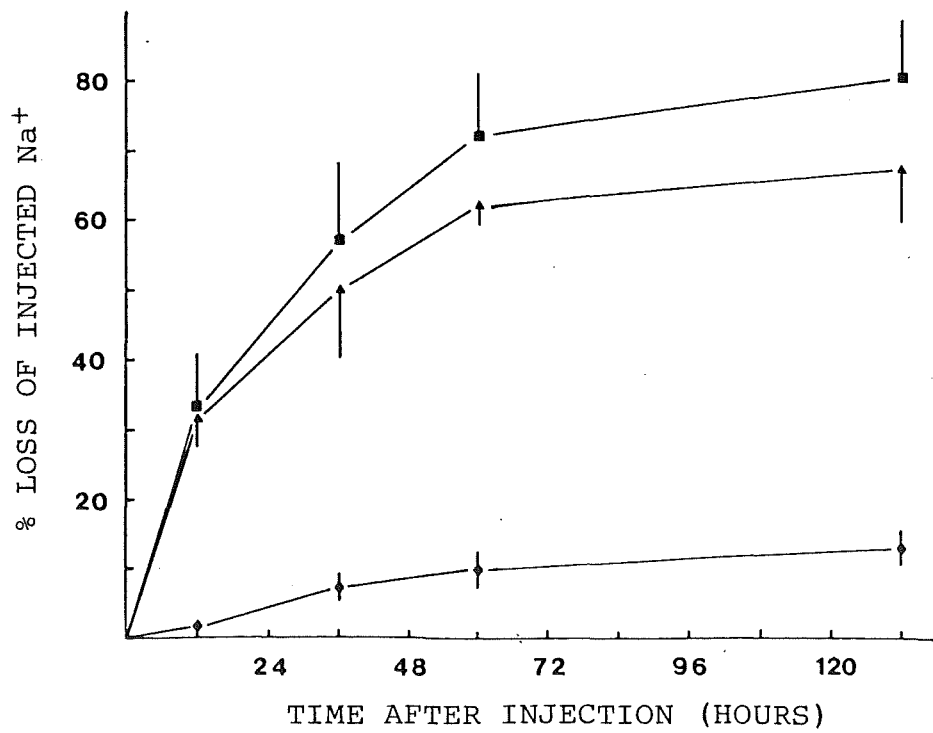
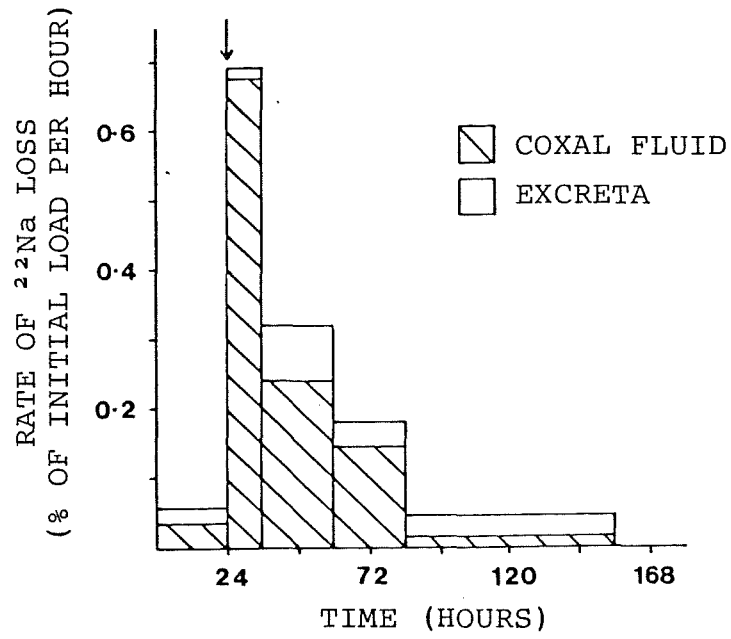
Despite the evidence for rapid regulation of the haemolymph Na concentration, little of the injected Na was excreted in the urine and faeces. In the 24 hours following injection, during which haemolymph regulation was achieved, only  $0.6 \mu\text{M}$  of Na were excreted and although Na excretion increased to  $1.76 \mu\text{M}$  in the following 24 hours it fell to  $0.5 \mu\text{M}$  in the subsequent 24 hours and was maintained at this rate for the remainder of the experiment (Figure II.15). Summarising, in the 96 hours following injection a total of only  $3.56 \mu\text{M}$  or 16% of the injected Na was excreted in the urine and faeces, yet there was no visual evidence of coxal excretion on the filter paper lining the experimental chambers.

As noted above, coxal fluid that had dried on the filter paper was difficult to locate with either ultra violet or normal light. Therefore, to test for coxal excretion following Na injection, the Na pool of the spiders was labelled with  $^{22}\text{Na}$  before injection. This allowed daily monitoring of the total  $^{22}\text{Na}$  loss of the spiders before and after injection by measuring the total  $^{22}\text{Na}$  content of the spiders. For this purpose the live spiders were encouraged into a small open-ended plastic tube. One end of the tube was sealed with parafilm and the spiders gently forced against the parafilm with a cotton dental bud. The orientation

Figure II.16    Rate of  $^{22}\text{Na}$  loss from spiders following injection with 60  $\mu\text{M}$  NaCl per gm spider. Arrow indicates time of injection. Height of bar represents total loss for a given interval., (n = 10).

Figure II.17    The excretion of Na following injection of 60  $\mu\text{M}$  NaCl per gm spider directly into the haemolymph. Vertical bars =  $\pm 1$  S.E.M., (n = 10).

■ ——— ■ Total excretion.  
▲ ——— ▲ Excretion by the coxal glands.  
◆ ——— ◆ Excretion in the excreta.



and position in the tubes were the same for all spiders. The tube was then introduced into the well counter with the spider at the bottom and  $^{22}\text{Na}$  content measured. The spiders exhibited no ill effects when released from the tubes. The  $^{22}\text{Na}$  content of the excreta and the filter paper left after the removal of the excreta, which will contain any coxal fluid excreted, were also measured daily, and the loss by these routes compared to the total loss. Rates of urine excretion and the actual Na content of the excreta and regions of coxal fluid were also measured, but changes in the haemolymph Na concentration were not.

Spiders were injected with  $^{22}\text{Na}$  and allowed 48 hours for equilibration and recovery. Normal rates of  $^{22}\text{Na}$  loss were measured for 24 hours, then experimental spiders were injected with 60  $\mu\text{M}$  NaCl per gm and control spiders with equivalent volumes of spider saline (see Section I.2.6 for composition). The total rate of  $^{22}\text{Na}$  loss and the subdivision of this into that lost through either anal or coxal excretion is shown in Figure II.16. Total loss increased twelvefold from  $0.057\% \text{ hr}^{-1}$  prior to injection, to  $0.69\% \text{ hr}^{-1}$  after injection, and although the rate subsequently decreased, it was 60 hours before it returned to pre-injection levels. Again it was significant that the  $^{22}\text{Na}$  recovered in the excreta was only a small portion of the total  $^{22}\text{Na}$  loss following injection. In the 12 hours immediately following injection, when total rate of  $^{22}\text{Na}$  loss was greatest, the rate of loss in the excreta was less than the loss rate before injection. In subsequent periods, the loss in the excreta did increase, a maximum of  $0.077\% \text{ hr}^{-1}$  being recorded 12 - 36 hours after the injection, but this was still small compared to the total loss in this period,  $0.32\% \text{ hr}^{-1}$ , and in the 60 hours following injection when total loss exceeded pre-injection levels, only 10.2% of the total  $^{22}\text{Na}$  excreted was recovered in the excreta. The remaining 89.8% was located on the filter paper as discrete regions of very high  $^{22}\text{Na}$  activity. Close inspection of these regions with both ultra violet and natural light did not provide any evidence of dry fluid. It is argued that these regions of  $^{22}\text{Na}$  activity correspond to spots of dry coxal fluid. Samples of coxal fluid collected, spotted and dried on filter paper were also not visible in either ultra violet or normal light. Furthermore, spiders fed salt loaded prey were observed with drops of coxal fluid suspended from the pre-oral region, and these drops were seen to be absorbed onto the filter paper lining the experimental chambers. Again the only means of locating these fluids was through their  $^{22}\text{Na}$  activity. The possibility that the spots of Na activity were actually dry mid-gut fluid

was discounted as the coloration and viscous nature of the mid-gut fluid rendered it clearly visible when dried onto filter paper.

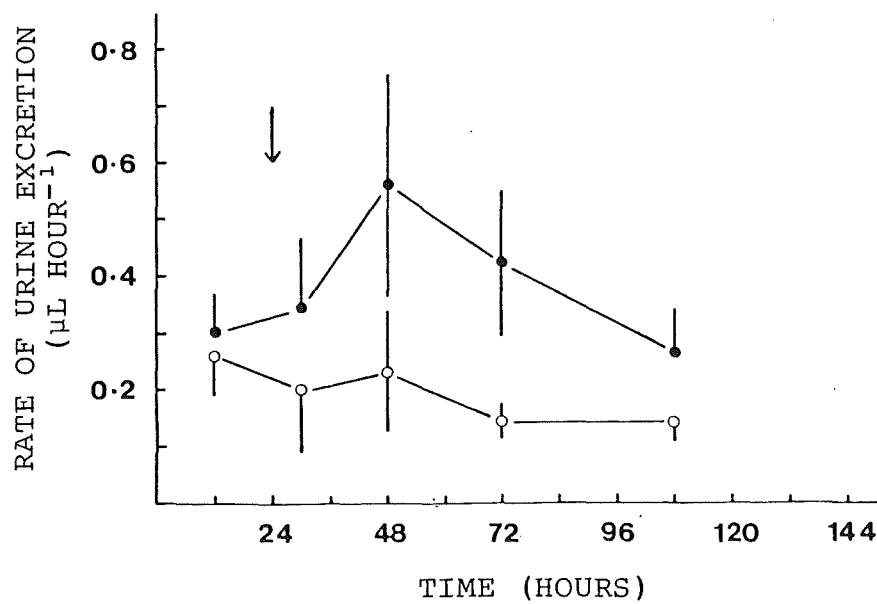
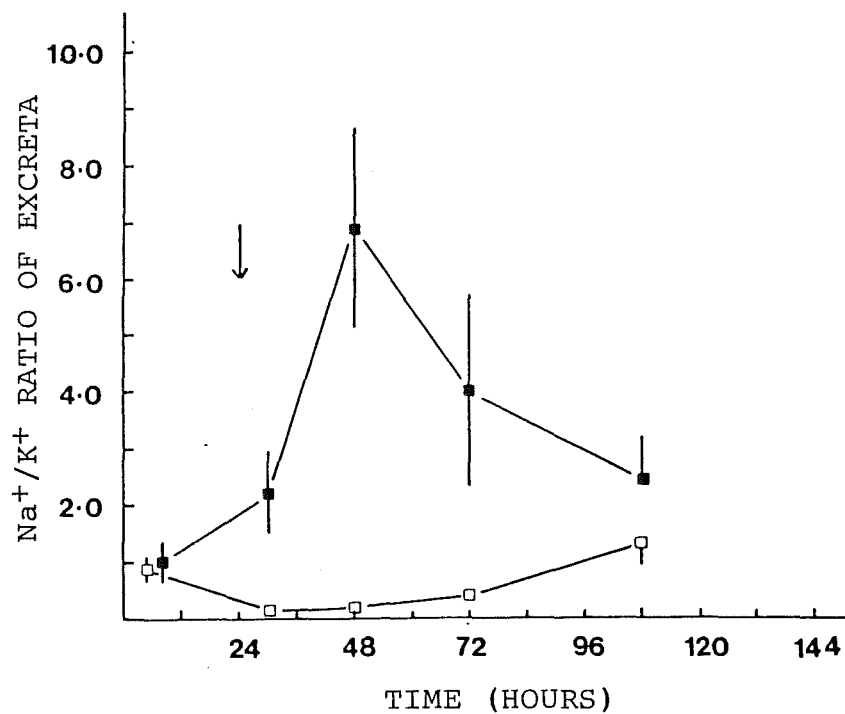
Attempts to confirm the involvement of the coxal glands by repeating the experiment with the coxal openings blocked were unsuccessful. The position of the coxal openings at the point of articulation of the coxal segments of the walking legs one and three, make it very difficult to guarantee the integrity of the seal placed over the openings. However, there are some pertinent observations from this attempt. Animals, in which the coxal openings were blocked with wax before injection with NaCl, were observed with dry salts at the edges of the blockage. In control animals, dried salts were never seen in the vicinity of the coxal openings. Furthermore, a number of individuals were observed with coxal fluid emerging from under the seal. Again this was not seen in control animals in which the coxal openings had been blocked.

The actual amounts of (cold) Na excreted by the coxal glands and the anal system following injection with 60  $\mu\text{M}$  NaCl per gm are shown in Figure II.17. These results confirm those obtained from measurements of  $^{22}\text{Na}$  loss, and show that in the 132 hours following injection 80% of the injected Na was excreted, but of this only 13% was recovered in the excreta, the remaining 67% was excreted by the coxal glands.

Although the anal system contributes little to the excretion on the injected Na, there are certainly indications of a response in this system to the injected salt load. The animals exhibit a brief diuresis during which the rate of urine excretion almost doubles in the period 12 - 36 hours after the injection, but thereafter urine excretion declines to pre-injection levels (Figure II.19). There is also a marked increase in the Na/K ratio of the excreta of the injected animals (Figure II.18). It is interesting, however, that this results from not only an increase in the Na concentration of the fluid, but also a decrease in the K concentration. Shown in Table II.6 is the composition of the stercoral fluid collected 24 hours after the injection into spiders of either 60  $\mu\text{M}$  NaCl per gm or equivalent volumes of saline. While the Na concentration increased from 41.9 to 103.6  $\text{mM l}^{-1}$ , the K concentration decreased from 35.5 to 24.1  $\text{mM l}^{-1}$ . The K concentration of the stercoral fluid of starved animals is so low that even small reductions can have a large effect on the Na/K ratio. In the absence of a decrease in the K

Figure II.18 The Na/K ratio of the excreta produced by *P. antipodiana* following injection of 60  $\mu$ M NaCl per gm spider directly into the haemolymph. Arrow indicates time of injection. Vertical bars =  $\pm$  1 S.E.M., (n = 10).  
Open symbols, control spiders injected with saline, (n = 10).  
Closed symbols, experimental spiders injected with NaCl, (n = 10).

Figure II.19 Rate of urine excretion by *P. antipodiana* following injection of 60  $\mu$ M NaCl per gm spider directly into the haemolymph. Arrow indicates time of injection. Vertical bars =  $\pm$  1 S.E.M.  
Open symbols, control spiders injected with saline, (n = 10).  
Closed symbols, experimental spiders injected with NaCl, (n = 10).





concentration the Na/K ratio would only have increased threefold. In such an artificial situation it is difficult to determine reasons for the reduction in the K concentration, however, with the increased urine excretion observed following injection with Na, the animals might be expected to limit the K loss.

Table II.6 The composition of stercoral fluid collected 24 hours after the injection of either 60  $\mu$ M NaCl per gm spider or, in the case of controls, equivalent volumes of saline ( $\bar{X} \pm \text{S.E.M.}$ ,  $n = 4$ ).

	Na (mM l <sup>-1</sup> )	K (mM l <sup>-1</sup> )	Na/K ratio
Na loaded spiders	103.6 $\pm$ 24	24.1 $\pm$ 8	5.8 $\pm$ 2.3
Saline injected controls	41.9 $\pm$ 19	35.5 $\pm$ 3.6	1.08 $\pm$ 0.38

## II.6 DISCUSSION

Prior to the observations on restrained feeding *P. antipodiana* reported here it was thought that the only fluids secreted into the prey by spiders were those produced by the midgut diverticula, gnathocoxal acini and the poison glands (Comstock, 1940; Millot, 1949; Snow, 1970; Legendre, 1978). Evidence presented here, however, demonstrates that coxal fluid is also secreted into the prey. The production of coxal fluid, in hydrated spiders fed normal prey, is limited to periods of feeding, and the fluid produced is transported to the pre-oral region where it mixes with the partially digested prey. Under conditions of Na loading, both by injection and modified diet, the operation of the coxal glands is modified and they exhibit a more positive excretory role. Following injection of Na loads into the haemolymph, the coxal glands are the major organs for the excretion of Na, and during feeding on Na loaded prey the coxal glands excrete greater quantities of Na into the prey debris and continue to excrete Na rich fluid after the completion of the meal. Similar responses are not observed following dietary K loading, the operation of the coxal glands of these animals resembling that of spiders fed prey of normal Na and K content.

Of the four fluids secreted into the prey (coxal fluid, midgut fluid, gnathocoxal fluid and poison), the coxal fluid is the only one that satisfies the four criteria proposed in the introduction, and therefore appears responsible, although maybe not alone, for the excretion of Na and K into the prey.

The fluid regurgitated via the mouth of *P. antipodiana* is in fact a combination of three fluids, the luminal contents of the midgut and foregut, digestive fluids secreted by the midgut diverticula, and small volumes of fluid secreted by the gnathocoxal acini, which mix with the other two fluids in the pre-oral region (Legendre, 1978). Although these fluids were excreted into the prey throughout feeding and so meet one of the proposed criteria, their initial composition was such that it does not appear that they could be responsible for the disproportionate excretion of Na and K into the prey debris (Section I). Furthermore, as feeding progresses the composition of the regurgitated fluid will be modified to a great extent by the contents of the gut lumen, and so reflect the composition of the ingested material.

Arguably, poison could be excreted into the prey throughout feeding. The chelicerae are repeatedly plunged into the prey and are active in the maceration of the prey tissue. However, extensive electrostimulation (Collatz and Mommsen, 1974) of the chelicerae of *P. antipodiana* produced only 300-500 nl of poison, requiring a Na concentration of the order of 10-20 M to account for the observed Na excretion.

On the other hand, coxal fluid was excreted throughout feeding, and the composition of the fluid was such that it could account for the disproportionate excretion of Na and K into the prey debris (Section I). Furthermore, the rate of Na and K excretion by the coxal glands was sufficient to account for the loss of Na and K in the debris. Sodium and K were excreted by the coxal glands at a rate of 9.6 and 2.6  $\mu\text{M hr}^{-1}$  (gm animal) $^{-1}$ , respectively. The loss of Na and K in the meal debris (6.5  $\mu\text{M Na}$  and 1.8  $\mu\text{M K}$ , values from Section I corrected for a 1 gm animal) occurred over a period of approximately three hours. Therefore, even allowing for reingestion during feeding, and brief interruptions of feeding, sufficient Na and K could be excreted by the coxal glands during the feeding period.

The reingestion of coxal fluid excreted into the prey is of some significance. Amaranth powder placed over the coxal openings during feeding stained the emerging coxal fluid bright red, and eventually the food material in the pre-oral region was also coloured red. Once all the powder was flushed from the openings, the intensity of staining of the pre-oral region was progressively reduced as feeding and coxal excretion continued, and dissection of the gut at the completion of the meal revealed amaranth in the gut lumen.

An estimate of the extent of reingestion can be obtained from comparison of rates of  $^{22}\text{Na}$  loss from spiders into prey (Section I) and the rate of Na excretion by the coxal glands. During feeding,  $^{22}\text{Na}$  was excreted into the prey by labelled spiders at a rate of 3% of the total  $^{22}\text{Na}$  content per hour (Section I). This is equivalent to 2.8  $\mu\text{M Na hr}^{-1}$  (gm animal) $^{-1}$ . The coxal glands excrete Na at a rate of 9.6  $\mu\text{M hr}^{-1}$  (gm animal) $^{-1}$ , therefore, 6.8  $\mu\text{M}$  of Na or 70% of the total excreted by the coxal glands is reingested.

The use of  $^{22}\text{Na}$  as a measure of total (cold) Na loss from the feeding spiders will be affected by a reduction in specific activity of

the  $^{22}\text{Na}$ , through ingestion and assimilation of (cold) Na from the prey. However, as the feeding periods were brief, and as only small quantities of (cold) prey Na were ingested relative to the total Na content of the spider, this effect will be small. The prey items provided to the spiders were small (0.08 - 0.10 gm) with a Na content of 8 - 10% (5 - 5.6  $\mu\text{M}$  Na) of the total Na content of the spiders. Furthermore, a maximum ingestion of one-third to one-half of the prey items occurred after one hour of feeding and during shorter periods of feeding significantly smaller portions of the prey were ingested. Therefore, the maximum reduction in specific activity would be of the order of 5% and in most cases it would be less than this.

The reingestion of 70% of the coxal fluid is of some significance to the water balance of the spider. The measured rate of total coxal excretion,  $74 \mu\text{l hr}^{-1} (\text{gm animal})^{-1}$ , is equivalent to the excretion of 7% of the total body weight or 35% of the haemolymph volume per hour. Assuming a close relationship between salts and water, reingestion reduces the rate of coxal fluid loss to  $22 \mu\text{l hr}^{-1} (\text{gm animal})^{-1}$ , or 10% of the haemolymph volume per hour. This in itself represents a considerable water loss. As demonstrated in Section I, water loss through coxal excretion into the prey debris was at a minimum, 45% of the maximum amount of water the spider could ingest with the food. This appears to be a considerable investment in terms of the water balance of the spider, particularly in view of the small return in water ingested from the prey, and most certainly contributes to the water deficit that *P. antipodiana* experiences during and following feeding (Section I).

Excretory organs in general are classified as secretion or filtration "kidneys" (Riegel, 1971). The major criterion for distinguishing between the two is the ability of the filtration kidneys to clear the polysaccharide inulin at a clearance ratio greater than or equal to one; e.g., the capillaries of the vertebrate glomerulus (Pitts, 1963); the coelomic sac of the crayfish antennal gland (Riegel and Kirschner, 1960); the pericardium of the octopus (Riegel, 1972); and the filtration membrane of the tick coxal gland (Kaufman *et al.*, 1982). The ability of the coxal glands of *P. antipodiana* to also clear inulin with a clearance ratio greater than one suggests the mechanism of fluid production within the coxal glands is by filtration and reabsorption. There is structural evidence of filtration membranes in spiders which supports this proposal (Buxton, 1913; Kummel, 1973) and filtration sites

have been demonstrated in the coxal glands of the closely related ticks (Kaufman *et al.*, 1982), scorpions (Rasmont, 1958) and oribatid mites (Woodring, 1973).

The inulin clearance ratio is generally considered indicative of the extent of water reabsorption in a filtration system (Davson, 1970). Recently, however, it was proposed by Binns (1969), and confirmed by Riegel *et al.* (1974), that clearance ratios slightly in excess of one could result from purely mechanical factors. Binns suggested that in *Carcinus maenas*, because of the large bladder volume, the lack of equilibrium between the haemolymph and bladder urine would result in an inulin concentration in the bladder fluid always exceeding that of the haemolymph, once the tracer-free urine had been emptied from the bladder. Mechanical factors such as this are unlikely to account for the inulin clearance ratio of the coxal glands of *P. antipodiana* exceeding one, as in the absence of a bladder the volume of the coxal glands is relatively small compared to the haemolymph volume, and the rate of excretion of the coxal glands is such that the fluid would not be retained within the coxal glands for a sufficient period of time. What cannot be discounted, however, is either secretion or absorption of inulin by the coxal glands. Small amounts of inulin secretion by the glands could account for the clearance ratio exceeding one and, alternatively, absorption of inulin by the coxal glands could mask even greater water reabsorption than is indicated. Recently it has been demonstrated that, in the lower vertebrates, inulin is reabsorbed after filtration (Braysher and Green, 1972; Bayenbach and Kirschner, 1976), and Greenaway (1981) has shown that inulin was absorbed from the urine of the crab *Holthuisana transversa*. However, in view of the particularly high rate of excretion of the coxal glands, greater absorption of water does not seem likely.

The salt stress imposed on *P. antipodiana* through diet and injection served two functions. Firstly, it demonstrated the homeostatic capabilities of *P. antipodiana* with regards to maintenance of haemolymph composition, and secondly, in stressing the homeostatic mechanism it emphasised the role of the two potential excretory systems, the coxal glands and the anal system, in the elimination of the excess salts.

Regarding haemolymph regulation, *P. antipodiana* exhibits an exceptional ability to regulate its haemolymph K concentration and a less efficient, but nevertheless, very effective control of haemolymph Na

concentration. The ingestion of approximately 50  $\mu\text{M}$  K, an amount equivalent to 90% of the total K content of the spiders, did not result in an increased haemolymph K concentration. Similarly, the ingestion of 70  $\mu\text{M}$  Na, equivalent to 110% of the total Na content of the spiders, resulted in an increase of only 30% in haemolymph Na concentration. Furthermore, within 24 hours of injecting 30  $\mu\text{M}$  Na ( $\text{gm animal}^{-1}$ ), equivalent to 42% of the total Na content of the spiders, the haemolymph Na concentration had returned to a level equivalent to that measured before injection.

This homeostatic control exhibited by *P. antipodiana* is certainly comparable to that reported for the insects. The oral administration of a very large dose of KCl to *Periplaneta americana* only increased the haemolymph K concentration 2.8 times (Tobias, 1948). Less efficient regulation was observed in *Rhodnius prolixus* (Ramsay, 1953). Small increases in the K content of the blood meal (1 part 171  $\text{mM l}^{-1}$  KCl : 3 parts normal blood) resulted in little change in the haemolymph K concentration. However, larger increases (3 parts 171  $\text{mM l}^{-1}$  KCl : 1 part blood) resulted in a general breakdown of the homeostatic mechanism and eventual death. A similar response was observed in *P. antipodiana* when K was injected directly into the spiders. The spiders were unable to tolerate the increased K concentration of the haemolymph and died before the regulatory mechanism could reduce the K concentration. *Drosophila melanogaster* demonstrated quite exceptional regulatory abilities surviving and developing on media which contained either 1.3 M KCl (Croghan and Lockwood, 1960) or 0.95 M NaCl (Waddington, 1959). Although there were significant changes in the Na and K concentrations of the haemolymph of these animals, the changes were slight with respect to the increased salt content of the media. It should be noted that regulation was measured in larvae which had been selected over several generations for their ability to survive on salt loaded media. *Schistocerca gregaria* exhibited efficient control of the haemolymph osmotic pressure, Na, K and Cl concentrations when starved at 70% R.H. with a hypertonic saline solution to drink (Phillips, 1964b). Again there were increases in the haemolymph ion concentrations but they were small relative to the dietary salt loading. Ramsay (1976) reported that a 13-fold increase in either the Na or K content of the diet of larval *Manduca sexta* resulted in only a twofold increase in the haemolymph ion concentrations. These animals were, however, raised from the second instar on a salt loaded diet and their regulatory capabilities measured during the final instar. They did not exhibit such efficient

salt regulation when normal larvae were injected with salt loads.

Both the coxal glands and the anal system of *P. antipodiana* showed a positive excretory response to the imposed salt loads, however, in the case of the coxal glands the response was limited to Na loading. In the absence of coxal involvement in the excretion of the excess K, there are two possible routes for its elimination; either absorption into the haemolymph and subsequent excretion by the Malpighian tubules or regions of the gut, or direct passage of much of the K through the gut to the stercoral pocket. Unfortunately, no estimate of the relative assimilation of K was possible, therefore, it is difficult to distinguish between the two routes. However, in view of the large K load encountered by the spiders, and the absence of any change in the K concentration of the haemolymph, it would appear that at least some of the ingested K passed directly through the gut.

The operation of the gut as a homeostatic mechanism has been proposed a number of times in the past for the insects. Ramsay (1953) suggested that the gut functioned in limiting the absorption of K into the haemolymph of *R. prolixus* and Gee (1975a) suggested a similar mechanism for *Glossina austeni*. Recently, Shaw and Stobbart (1972) demonstrated that for *S. gregaria* and *Periplaneta americana* under conditions of survival on a dry diet when the material ingested formed a hyperosmotic fluid in the gut, solutes and water could not be assimilated into the haemolymph then excreted by the Malpighian tubules and rectum, unless the haemolymph osmotic pressure rose markedly. As little increase in the haemolymph osmotic pressure does occur in these animals under these conditions, Shaw and Stobbart proposed that the midgut acted as a homeostatic device, limiting the absorption of ions and water. They proposed that the material passed directly to the rectum which then exerted its full osmoregulatory powers and extracted osmotically free water from the meal.

An important difference between the conditions described by Shaw and Stobbart and the conditions encountered by *P. antipodiana* was that *P. antipodiana* had access to free water. With free water to drink, both *S. gregaria* and *Periplaneta americana* could maintain their haemolymph composition even with the assimilation of ions and subsequent excretion. Therefore, arguably so may *P. antipodiana*, and it is notable that salt loaded spiders maintained a much higher rate of urine excretion than control spiders which would have required the animals to drink greater

quantities of water. If this is the case, a fine coordination of assimilation and excretion would be required to achieve the regulation observed.

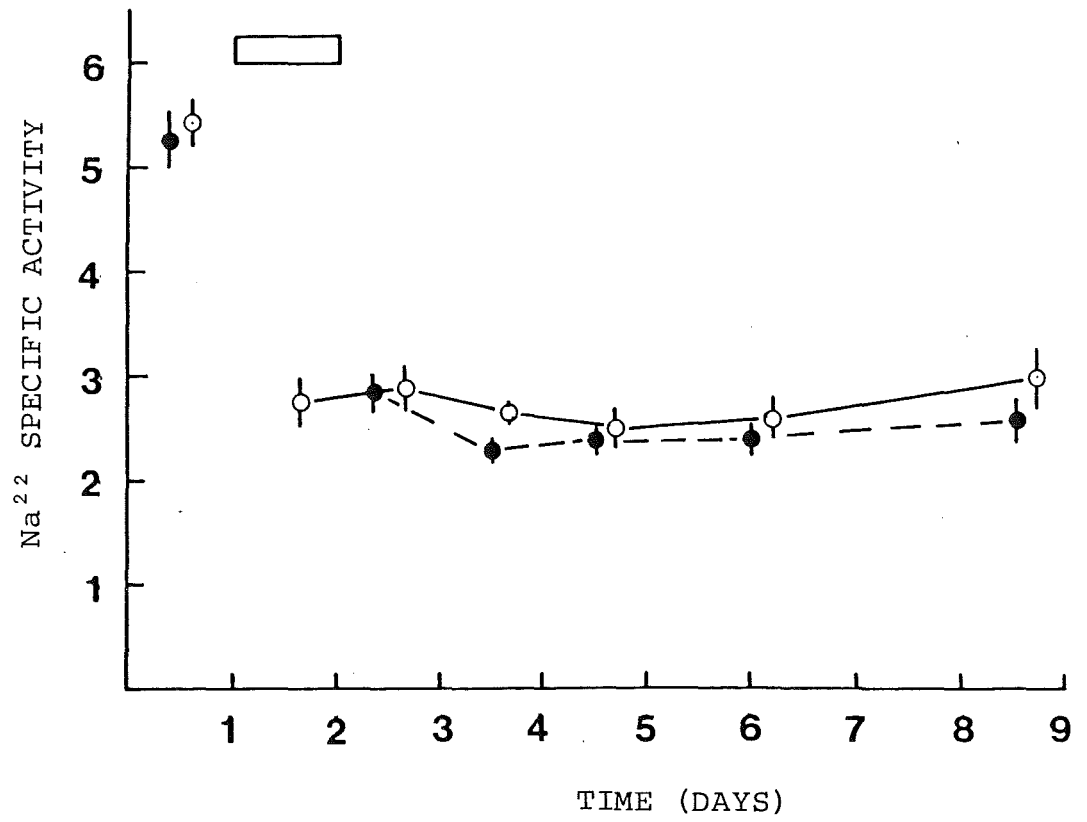
Tissue regulation of haemolymph K concentration could assist this process. Tobias (1948) noted that following K loading of cockroaches K was concentrated in the nervous tissues and muscles, and Ramsay (1953) suggested that this may involve tissue regulation of the haemolymph. Recently it was proposed that the fat body of the cockroach functions as an ion sink and is active in the osmotic and ionic regulation of the haemolymph (Mullins, 1974; Mullins and Cochran, 1974). There is evidence that K is concentrated in the tissues of *P. antipodiana*. The total K content of fed *P. antipodiana* increased significantly with little change in the haemolymph K concentration but marked changes in the tissue concentration (Section I). A system such as this operating in concert with the excretory system may achieve the control of haemolymph K observed in *P. antipodiana*.

Although no estimate of the relative assimilation of K was possible, there was evidence of significant assimilation of Na into the haemolymph of animals provided with Na loaded food. Sodium loaded spiders were labelled with  $^{22}\text{Na}$  prior to feeding. If significant amounts of (cold) Na from the prey passed directly through the gut the specific activity of the excreta would be lower than that of the haemolymph. However, if the (cold) prey Na was assimilated into the haemolymph then excreted, the specific activity of the haemolymph and excreta would be similar. The specific activity of the haemolymph of Na loaded animals was not measured, but that of the coxal fluid, which is derived from the haemolymph and so should be representative of the haemolymph, was. Significantly, following ingestion of Na loaded prey the specific activity of the coxal fluid and the excreta decreased to a similar extent (Figure II.20), indicating that most of the Na was assimilated into the haemolymph before excretion by both the anal system and the coxal glands. Thus the anal system is capable of responding to increased haemolymph Na concentrations and excreting the excess ions.

In view of this it remains to be explained why the anal system was so ineffective in the removal of Na following the injection of Na directly into the haemolymph. In Section I it was shown that post prandial diuresis was responsible for the elimination of ions ingested with a meal and it is worth noting that, following the ingestion of salt loaded prey,



Figure II.20      The specific activity of  $^{22}\text{Na}$  in the excreta and coxal fluid of spiders fed Na loaded prey. Horizontal bar represents feeding period. Vertical bars =  $\pm 1 \text{ S.E.M.}$  Open symbols, excreta. Closed symbols, coxal fluid, (n = 10).



the extent and the duration of this diuresis was increased. However, following injection with  $60 \mu\text{M NaCl (gm animal)}^{-1}$ , a salt load approximately equivalent to that ingested during dietary salt loading, only a small diuretic response was observed. In insects it has been demonstrated that post prandial diuresis is under hormonal control (Maddrell, 1963; Berridge, 1966b; Pilcher, 1970; Gee, 1975b) and the lack of a significant diuretic response by the Na injected spiders may reflect the absence of the necessary stimuli which elicit the release of a possible diuretic hormone. The relatively small increase in the Na concentration of the stercoral fluid of these spiders may also result from the inactivity of the excretory apparatus. Berridge (1966) demonstrated that the non-diuretic Malpighian tubules of *Dysdercus fasciatus* did not secrete fluid until stimulated with hormone.

It is interesting that the coxal glands responded equally to injected or dietary Na loading, suggesting that the control of the coxal glands differs from that of the anal system. The speed of response of coxal excretion to feeding suggests a possible neural control, and this may be mediated via the coxal openings, closure of the openings generating back pressure within the coxal glands which prevents filtration. Kaufman *et al.* (1982) demonstrated that cannulation of the coxal openings of the tick, *Ornithodoros moubata*, doubled the rate of coxal excretion.

The response of the coxal glands to salt loading, although limited to Na loading, suggests that the coxal glands function as excretory organs. Following the injection of Na loads directly into the haemolymph the coxal glands were the major route for the elimination of the injected Na, and during dietary Na loading the quantity of Na excreted into the prey by the coxal glands was increased and the coxal glands continue to excrete coxal fluid after the completion of the meal. This proposed excretory role of the coxal glands receives further support when their functioning under conditions of water deprivation and rehydration is considered (Section IV). During water deprivation, in response to small increases in the osmotic pressure and Na concentration of the haemolymph there were marked increases in the osmotic pressure and Na concentration of the coxal fluid excreted into the prey. Furthermore, during rehydration of spiders, following periods of water deprivation, the coxal glands were active in the excretion of excess ions ingested by the spiders during the period of water deprivation. A second example of coxal excretion at times other than feeding.

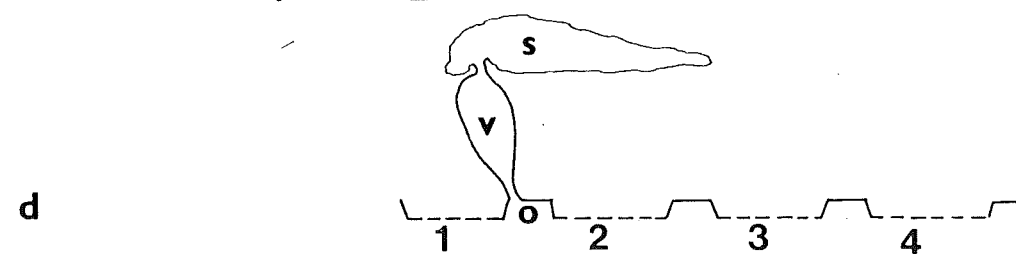
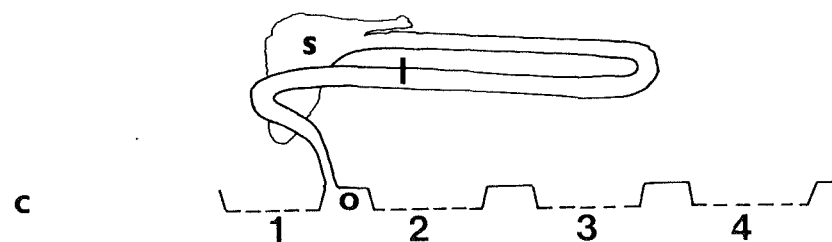
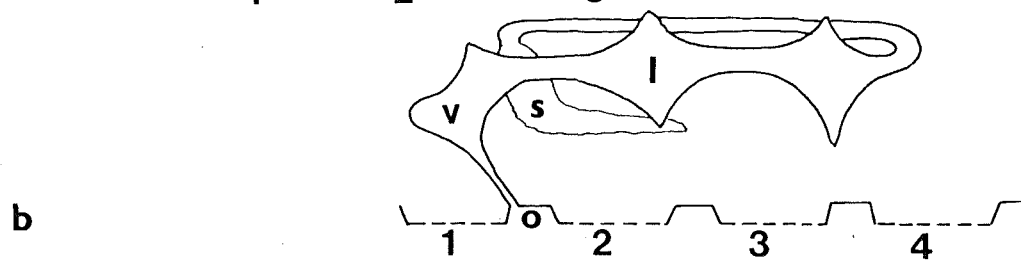
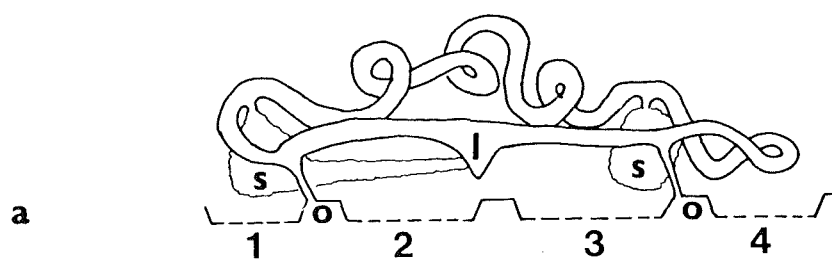
The primitive function of the coelomoduct derived organs, such as the coxal glands of spiders, is thought to be ionic and osmotic regulation (Goodrich, 1945; Clarke, 1979), and there is considerable evidence that representatives of these organs in the present day arthropods retain this function, e.g. the coxal glands of *Limulus polyphemus* (Mangum and Mauro, 1980; Towle *et al.*, 1980), the coxal glands of the argasid ticks (Lees, 1946; Frayha *et al.*, 1974; Kaufman *et al.*, 1980), the coxal glands of the oribatid mites (Woodring, 1973) and, with some reservations (Shaw, 1964), the antennal and maxillary glands of Crustacea (Parry, 1955; Burger, 1957; Lockwood and Riegel, 1969; Riegel and Cook, 1975). However, the excretory function of the coxal glands of *P. antipodiana* has to be reconciled with the close association that coxal excretion has with feeding. Hydrated animals fed on prey of "normal" salt content, generally only excrete coxal fluid during feeding, and this fluid is transported forward to the pre-oral region where much of it is reingested. I suggest that the coxal fluid aids in the feeding process of *P. antipodiana* which, like all spiders, digests its prey extra-orally and then ingests the semi-liquified food (Legendre, 1978). The partially digested semi-liquid prey is ingested by suction generated in the pumping stomach and the oesophagus. The role the coxal glands play in this process is that of a "pseudo salivary" gland providing a continual supply of fluid which suspends the partially digested food material and aids the operation of the suction process by continually priming the pump.

It is interesting that, among the spiders as a group, there is a trend towards the reduction in complexity of the coxal glands and a concentration of the remaining elements in the anterior region of the cephalothorax (Buxton, 1913 and Figure IV.21). In primitive mygalomorph spiders, such as *P. antipodiana*, the coxal gland consists of two saccules, an extensive tubular labyrinth system and two outlets. In the more advanced Araneae, the anterior saccule only is retained, and there is a gradual reduction and simplification of the labyrinth system, until in the most advanced web-spinning spiders the labyrinth is reduced to a simple bladder. Millot (1949) proposed that these changes were indicative of the obsolescence of the coxal glands, their excretory function having been taken over by the Malpighian tubules. Buxton (1913) had earlier suggested that the reduction in complexity was a case of simplification with increased efficiency of the remainder, since the saccule, which is the site of fluid production, is retained in good working order. It may be that both authors were, in fact, correct.

Figure II.21      Comparison of the different forms of coxal glands present in the spiders (after Buxton, 1913).

- (a) Mygalomorph spiders.
- (b) Hunting spiders (Lycosidae, Thomsidae).
- (c) Sicariidae, Dysderidae.
- (d) Web spinners (Epeiridae, Theridiidae).

Numbers 1 - 4 represent positions of the walking legs.  
s = sacculle, l = labyrinth, v = vesicular bladder,  
o = coxal opening.



Goodrich (1945) noted that among the arthropods there is an increasing trend for what were, primitively, excretory coelomoduct organs to be reduced in numbers in the adult, and specialised as "salivary glands" in the head region, excretory glands in the mid trunk region and genital ducts in the posterior region. The observed changes in the arrangement and complexity of the coxal glands of spiders may well be an example of the modification of a coelomoduct-derived excretory organ into a "salivary gland", the definite excretory function of the coxal glands of *P. antipodiana* simply being a virtue of the fact that *P. antipodiana* is a primitive representative of the spiders, and thus the process has not completed its course in this animal.

## II.7 SUMMARY

1. The source of Na excretion into the prey during feeding by *Porrhothele antipodiana* was investigated.
2. The coxal glands were found to excrete fluid during feeding. In spiders fed prey of "normal" salt composition the excretion of coxal fluid was limited to the period of feeding.
3. The fluid produced by the coxal glands during feeding is transported forward to the mouth along grooves on the ventral surface of the cephalothorax.
4. Coxal fluid is produced at a total rate of  $72 \mu\text{l hr}^{-1} \text{ gm animal}^{-1}$ . Approximately 70% of the coxal fluid is re-ingested during feeding, implicating the coxal fluid in the feeding process of the spider.
5. The coxal fluid is hypo-osmotic to the haemolymph with a high Na concentration relative to K. It is argued that coxal excretion is responsible for the elimination of Na and K in the prey debris.
6. The possible role of the coxal glands as an excretory organ was studied and compared to the functioning of the anal system.
7. Salt stress was imposed by modification of the diet and injection into the haemolymph.
8. The coxal glands responded only to increased Na loads. Following Na injection the coxal glands were the major route for the elimination of Na and excreted when the animals were not feeding. Following dietary Na loading glands continued excretion after the completion of the meal.
9. The anal system responded to both increased Na and K loads, excreting both salts at markedly increased rates. A feature of this response was a marked increase in the rate of urine production. The anal system showed little response to injected Na loading.
10. *P. antipodiana* exhibited an excellent ability to regulate its haemolymph K concentration and a less efficient, but nevertheless, very effective control of haemolymph Na.



III.    TRANSPIRATIONAL WATERLOSS IN *PORRHOTHELE ANTIPODIANA*, AND  
THE EFFECTS OF DEHYDRATION ON THE COMPOSITION OF THE  
HAEMOLYMPH.

### III.1 INTRODUCTION

Desiccation due to the dryness of the environment is a major problem facing any terrestrial animal, particularly the invertebrates which, because of their small size, have a large surface to volume ratio. Therefore, it is not unexpected that the success of the insects in the terrestrial environment can be attributed in large measure to the evolution of efficient mechanisms for the conservation of water (Phillips, 1970; Berridge, 1970), and so we see in the terrestrial insects a highly impermeable cuticle (Wigglesworth, 1945; Beament, 1964; Ebeling, 1974; Gilby, 1980), mechanisms for reducing respiratory waterloss (Mellanby, 1934; Jackovlev and Kruger, 1953; Bursell, 1957, 1959, 1974; Miller, 1964a, b; Loveridge, 1968a, b; Krasfur, 1971a, b) and the ability to produce excreta which are hyperosmotic to the haemolymph (Wigglesworth, 1931; Ramsay, 1952, 1955, 1964; Phillips, 1964, 1970).

The spiders are representatives of the other highly successful terrestrial arthropod group and, although less thoroughly investigated, they appear to have evolved mechanisms similar to those of the insects for conserving water. Rates of cuticular waterloss of the spiders (Davies and Edney, 1952; Cloudsley-Thompson, 1957; Stewart and Martin, 1970; Seymour and Vinegar, 1973; Humphreys, 1975) are as low of those of insects of comparable size, and the structure of the cuticle of the spiders (Barth, 1969, 1970; Hadley, 1981) resembles that of insects, waxes being present which appear to confer the observed low permeability of the cuticle to water (Hadley, 1978). Spiracular closer mechanisms, or valves, have been described on the booklung openings of a number of spiders (Hazelhoff, 1926; Kastner, 1924; Millot, 1949; Robinson and Paim, 1969), and the valves respond to  $\text{CO}_2$  in a similar manner as the closer mechanisms of the spiracles of insects, opening in response to elevated  $\text{CO}_2$  concentrations in air (Robinson and Paim, 1969). Furthermore, waterloss has been shown to increase in a number of spiders when they were exposed to dry air and  $\text{CO}_2$  mixtures (Davies and Edney, 1952; Cloudsley-Thompson, 1957; Stewart and Martin, 1970), suggesting that respiratory waterloss is normally reduced by the spiracular valves. Information concerning waterloss through silk production and excretion of spiders is limited, although Ueda (1974) presented evidence that some spiders produce hyper-osmotic stercoral fluid during dehydration, and Witt *et al.* (1968) suggested that water is extracted from the silk of spiders before the silk is drawn from the spinnerets.

In spite of these mechanisms for the conservation of water in spiders, transpiration was shown to be a major source of waterloss from the spider *Porrhothele antipodiana* (Section I), and in this section measurements of the various components of transpirational waterloss of *P. antipodiana* are reported. Also reported are the effects of dehydration on the composition of the haemolymph of *P. antipodiana*.

Terrestrial animals inevitably encounter periods during which they experience waterlosses which cannot be replaced by drinking or feeding. Among the terrestrial arthropods varying degrees of regulation of the haemolymph composition during these periods of water shortage are observed. The terrestrial crustaceans show no regulation of the haemolymph composition during dehydration, and increases in the haemolymph osmotic pressure and ion concentrations correspond to values predicted from measurements of the reduction of haemolymph volume or water content of the animals (Bliss, 1968; Greenaway and MacMillen, 1978; Harris and Kormanik, 1981). In the insects, however, some form of regulation is usually apparent (Edney, 1966, 1968; Djajakusumah and Miles, 1966; Wall, 1970; Okasha, 1973; Nicolson *et al.*, 1974; Laird and Winston, 1975; Riddle *et al.*, 1976; Broza *et al.*, 1976; Coutchié *et al.*, 1979; Nicolson, 1980; Fielding and Nicolson, 1980), and recently a mechanism to account for the haemolymph regulation observed in the cockroach *Periplaneta americana* has been proposed (Mullins, 1974; Mullins and Cochran, 1974; Tucker, 1977a,b, c,d; Hyatt and Marshall, 1977). As far as I am aware, however, no previous measurements of the effects of dehydration on the haemolymph composition of spiders have been reported.

### III.2 METHODS AND MATERIALS

Adult female *P. antipodiana* were used at all times. Details of the collection and general maintenance of the spiders are given elsewhere (Section I).

#### III.2.1 Dehydration and Measurement of Waterloss

Prior to measurements of waterloss, all spiders were treated in the following manner. The animals were removed from the culture and placed in individual clean plastic containers (15×12×6.5 cm) with water but no food for two days. Immediately before waterloss measurements they were transferred to clean containers without water, and kept in these containers for five hours at room humidity (50 - 70% R.H.) and 20°C to remove any water adsorbed onto the cuticle or loosely bound to the cuticular protein (Hadley, 1970).

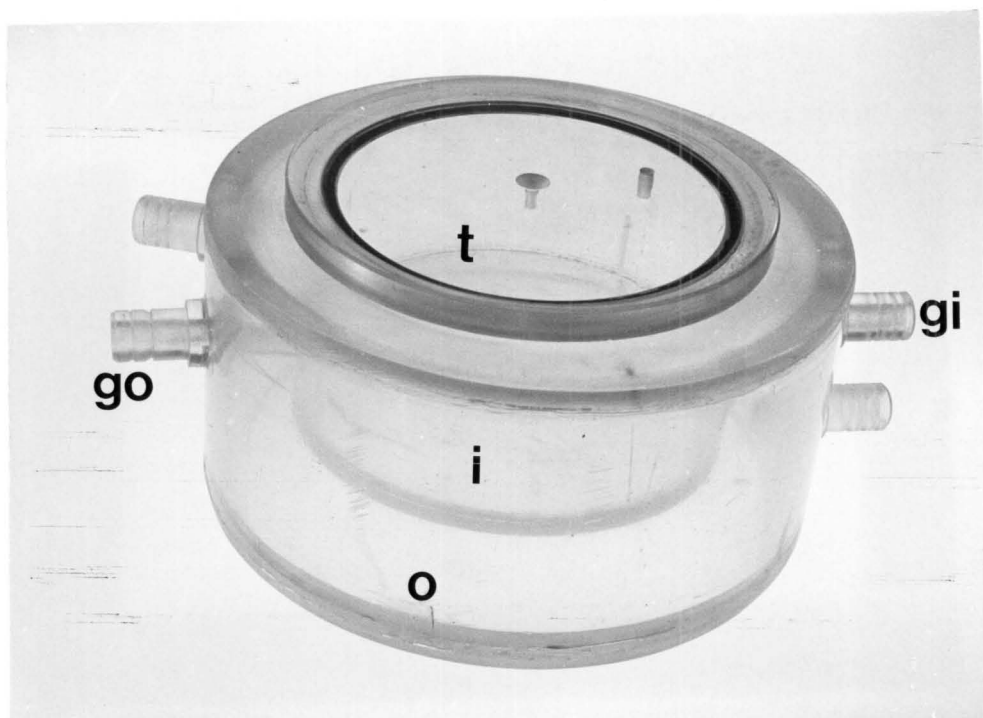
During waterloss measurements spiders were placed in individual pre-weighed glass containers with a plastic gauze top, and dehydrated in vacuum glass desiccators over silica gel. Air in the desiccators was circulated continuously with an electric fan sealed into the lid of the desiccators. The relative humidity of the air, which was always less than 10%, was measured with cobalt thiocyanate paper (B.D.H. Laboratory Reagents) and compared to standards from a Lovibond Humidity Test Kit (Tintometer Ltd., London). The desiccators were placed in constant temperature cabinets (Controlled Environments, Canada), and maintained at  $20 \pm 0.5^\circ\text{C}$  with a 12 hour illumination cycle. The weight of the containers plus the spiders was measured with a Mettler H10 balance, accurate to 0.1 mg. Waterloss was assumed to be equivalent to weight loss.

As the spiders were starved throughout the experiments, the only source of excretory waterloss should have been urine excretion via the anus. Coxal excretion by *P. antipodiana* is restricted to periods of feeding (Section II) and rehydration (Section IV). When measured, the rates of urine production were determined as described in Section I.

Following periods of dehydration, spiders were rehydrated by providing free water for drinking. During rehydration, relative humidity

Figure III.1    Experimental chamber employed for varying the gas composition to which spiders were exposed during measurements of spiracular valve movements.

i., inner gas chamber; o., outer water jacket for temperature control; gi and go., gas inlet and outlet; t., removable gas tight lid.



was not strictly controlled. The spiders were transferred from the desiccators to clean containers, which had cotton dental buds, soaked in tapwater, placed in small plastic troughs located on the floor of the containers. These containers were placed on trays in the constant temperature cabinet and kept at a temperature of  $20 \pm 0.5^\circ\text{C}$  and a relative humidity which varied between 50% and 70%.

When humidities other than 0% R.H. were required, saturated salt solutions as described by Winston and Bates (1960) were used. The salt solutions were placed in the bottom of glass chambers (25 cm in diameter and 18 cm deep) and the spiders, in individual containers, were placed on wire trays suspended above the salt solutions. The air in the glass chambers was circulated by an electric fan, and the chambers were kept in the constant temperature cabinets at  $20^\circ\text{C}$ .

### III.2.2 Measurement of the Surface Area of the Spiders

The surface area of the animals was determined from the equation  $S = kW^{0.66}$ , where  $S$  = the surface area,  $W$  = the weight of the spiders and  $k$  is a constant. The value of  $k$  was determined for five spiders. The spiders were weighed, completely eviscerated, their integument cut into small flat pieces and mounted on glass microscope slides. The outline of each piece of cuticle was drawn onto paper with the aid of a camera lucida, and the pieces of paper cut out and weighed. From a relationship between the weight and area of the paper, the surface area of the spider was calculated. An average value of  $11.9 \pm 0.85$  ( $\bar{X} \pm \text{S.E.M.}$ ,  $n = 5$ ) was obtained for the constant  $k$ . This compares with values of 12 reported for the genus *Ciniflo* (Cloudsley-Thompson, 1957), 12.3 for *Lycosa amentata* (Davies and Edney, 1952) and 12.8 for *Dugesiella hentzi* (Stewart and Martin, 1970).

### III.2.3 Recording of Spiracular Valve Movements

Each spider was immobilised on its dorsal surface against a small block of wax by applying melted beeswax across the femur of each walking leg. This allowed the spider to flex its tibia but not move its cephalothorax. The abdomen was prevented from moving by placing a thin strip of cellotape across the posterior half of the abdomen. The cellotape was held firmly on each side of the abdomen and served to hold

the abdomen in a small depression in the beeswax mount. The mount plus the attached spider was placed in an airtight, 4 cm deep, circular chamber constructed from a cut-down piece of 6 cm diameter perspex tubing (Figure III.1). The top of the chamber was sealed with a circular piece of 1.25 cm thick perspex milled to give a 0.6 cm lip which slotted into the gas chamber. An 'O' ring on the edge of the lip provided a gas-tight seal. The gas chamber was surrounded on all sides, except the top, with a water jacket through which water was circulated continuously, and its temperature altered to maintain the temperature in the chamber at 20°C. A thermistor probe inserted through the lid of the chamber monitored the chamber temperature continuously.

Gas mixtures were made by proportional flow using calibrated flowmeters (Gilmont Flowmeter Kit, F6500). The mixtures were bubbled through water at 20°C to ensure they were at the right temperature, and to maintain the humidity as high as possible in the chamber. Gases were passed through the chamber at a rate of 700 ml min<sup>-1</sup>.

Sequence of exposure to various gas mixtures was randomised to avoid cumulative effects, and the spiders were allowed 15 minutes recovery in normal air between measurements.

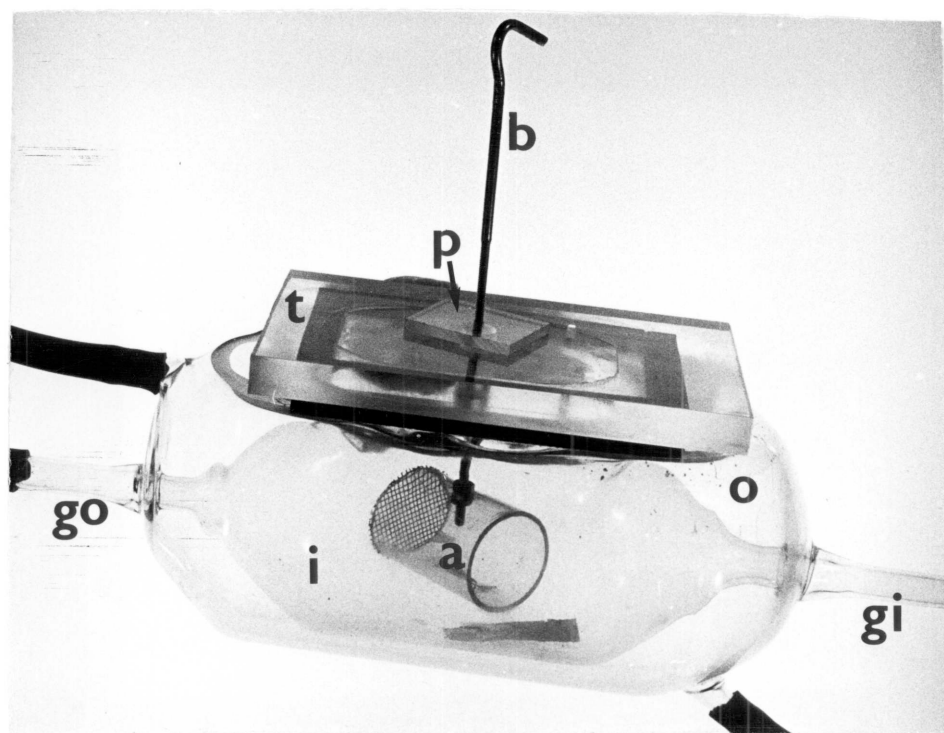
Several methods of recording spiracular valve movement have been reported. The method employed was a modification of the technique used by Schneiderman (1960). The gas chamber with the spider was placed on the stage of a binocular microscope (Wild Heerbrugg Ltd.) with an electronic ocular micrometer attached. The hairline of the micrometer was focused on the edge of the spiracular valve and its position adjusted to follow the movement of the valve as it opened and closed. The reference cross hairs of the micrometer indicated the position of the valve when it was closed. The movements of the hairline were recorded with a pen recorder attached to the ocular micrometer via a voltmeter. The tracings provided a record of spiracular movement, the ordinate indicating the extent of opening and the abscissa the duration.

#### III.2.4 Measurement of Waterloss During Sustained Opening of Spiracular Valves

The rate of waterloss was measured in a specially constructed desiccation chamber which allowed the spider to be weighed at regular



Figure III.2    Experimental chamber employed for the measurement of weight changes in animals exposed to elevated CO<sub>2</sub> levels in dry air. a., animal chamber; i., inner gas chamber; o., outer water jacket; gi and go., gas inlet and outlet; t., removable gas tight lid; b., brass weighing hook; p., perspex support for animal chamber between weighings.



intervals without disturbance of the spider or alteration of the relative humidity and gas composition in the chamber (Figure III.2). The spider was placed in a small perspex tube (4.5 cm long, 2.7 cm in diameter) suspended from a brass hook which passed through a hole in the lid of the chamber. This hole was sealed by a small square of perspex threaded onto the brass hook, the perspex also acting to support the hook and the animal's chamber between weighings. To weigh the spider, the gas flow was turned off and the brass hook raised and hung from the suspension arm of a Mettler H10 balance. Each measurement required from 10 - 20 sec.

The temperature in the chamber was monitored at all times with a thermistor probe inserted through the lid. Water was continuously circulated through a water jacket surrounding the chamber and the temperature of the water altered to ensure that the temperature in the chamber was maintained at  $20 \pm 0.5^\circ\text{C}$ .

Compressed air and  $\text{CO}_2$  were mixed by proportional flow (Gilmont Flowmeter Kit, F6500) to give a final composition of 90% air 10%  $\text{CO}_2$ . This mixture, or normal air, was passed through indicator silica gel and several coils of copper tubing emersed in a water bath at  $20^\circ\text{C}$ , then through the desiccation chamber at a rate of  $750 \text{ ml min}^{-1}$ .

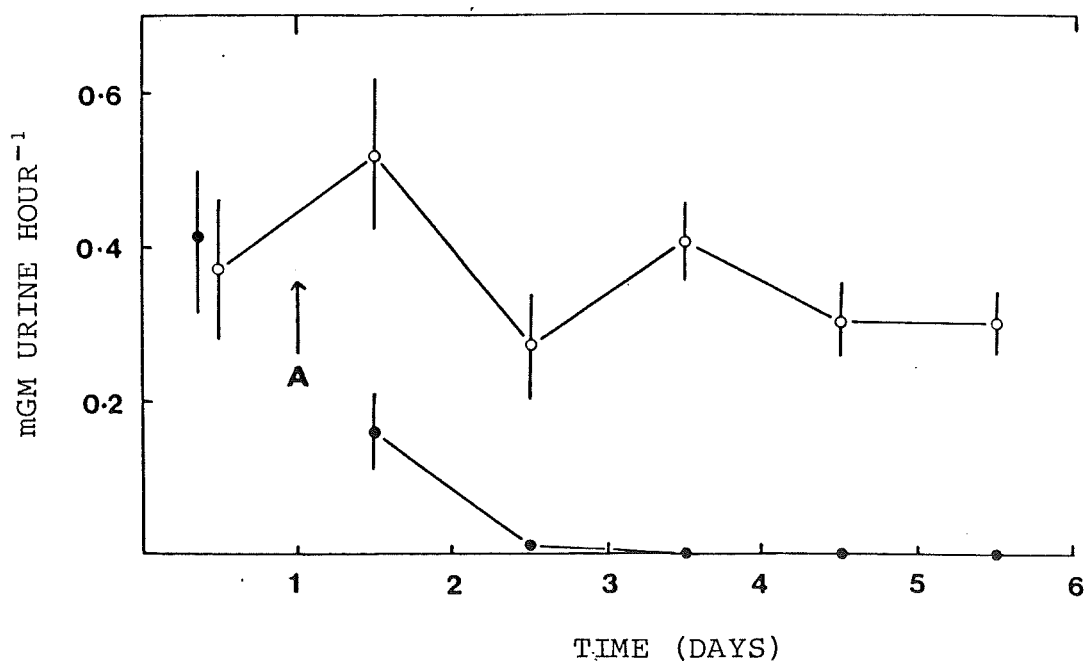
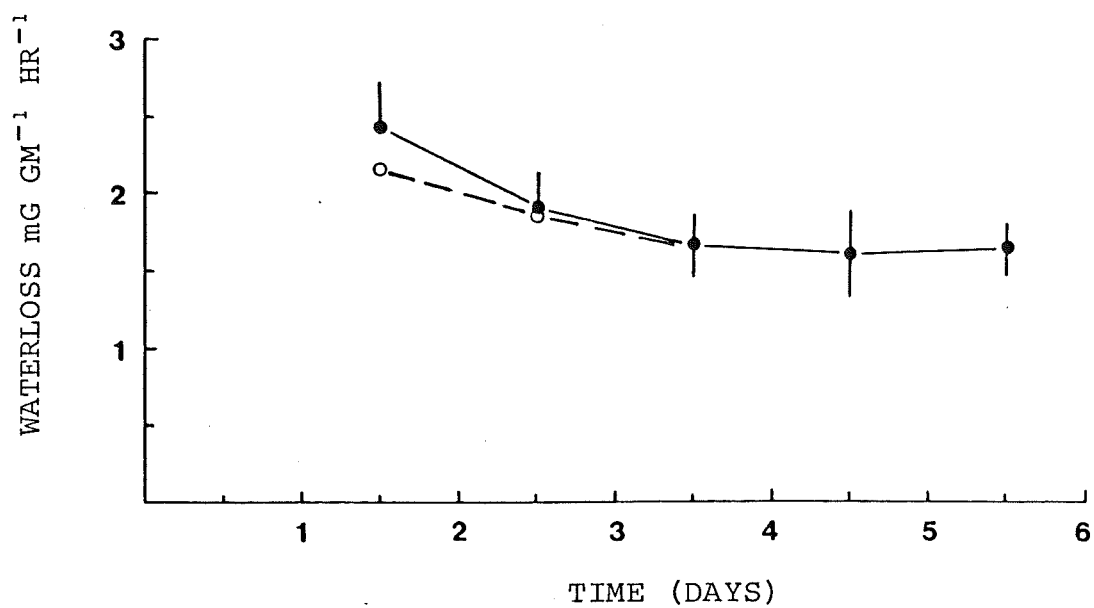
Spiders were initially exposed to dry air at  $20^\circ\text{C}$  for three hours, then dry air plus 10%  $\text{CO}_2$  for a further four hours. The gas composition was then returned to normal air and the waterloss rate measured until it returned to rates equivalent to those before exposure to 10%  $\text{CO}_2$ . Spiders which defaecated during the experiment were not included in the results.

### III.2.5 Collection and Analysis of Excreta and Haemolymph Samples

Haemolymph samples were collected and their osmotic pressure, Na, K and Cl concentrations measured as described in Section I.2. Experiments in which the Na and K content of the excreta were to be determined were carried out in small glass containers lined with Whatman 542 Quantitative filter paper (Section I.2). The ion content of the dried excreta on the filter paper was determined as in Section I.2.

Figure III.3 Time course of changes of weight specific rate of waterloss of *P. antipodiana* during dehydration in dry air at 20°C. Dotted line represents water lost by transpiration alone, i.e., total loss minus loss through defaecation. Vertical lines =  $\pm 1$  S.E.M., n = 10.

Figure III.4 The effect of dehydration on the amount of urine excreted by *P. antipodiana*. Open symbols, control animals provided with water, n = 10. Closed symbols, experimental animals dehydrated in dry air at 20°C from point A, n = 10. Vertical lines =  $\pm 1$  S.E.M.



### III.3 RESULTS

#### III.3.1 Total Rate of Waterloss and Urine Production

The weight specific rate of waterloss of *P. antipodiana* dehydrated at 0% R.H. and 20°C for six days is shown in Figure III.3. Total waterloss declined progressively over the first three days of dehydration until reaching a stable rate of between 1.6 and 1.7 mgm gm<sup>-1</sup>hr<sup>-1</sup> in the third day. Associated with this decline in waterloss was a reduction in the volume of urine produced by the animals (Figure III.4). During the first day of dehydration the mean rate of urine production (0.16 mgm gm<sup>-1</sup>) was less than half the rate measured prior to dehydration and also half the rate observed in hydrated controls. Urine production ceased completely after two days of dehydration and no further urine was produced during the remaining three days of the experiment. Even this complete cessation of urine production observed in dehydrated spiders could account for only 27% of the reduction in the rate of total waterloss. Therefore, other compensatory mechanisms must be involved in reducing the waterloss.

#### III.3.2 Respiratory Waterloss

Attempts to determine the rate of water loss associated with respiratory exchange by comparing the rate of waterloss of living and dead spiders were unsuccessful. Spiders were allowed to construct a web in the experimental chambers, then they were killed with coal gas and their booklungs blocked with nail varnish before they were transferred back into the centre of their own webs, and placed in the desiccators. Live control spiders were allowed to construct a web in the experimental chambers, then a small amount of nail varnish was applied to the abdominal cuticle and dried before placing the spiders in the desiccators. Total waterloss for the two groups was measured over three hours. Dead spiders with blocked booklungs were found to have a greater rate of water loss than live controls (Table III.1).

The experiment was repeated with spiders which were not killed before blockage of the booklungs. Live animals were gently restrained and the booklungs sealed with a small patch of nail varnish. They were then transferred to the desiccators in their own webs and waterloss measured for three hours. After measurement of waterloss, the spiders

were removed from the desiccators, the nail varnish removed from the spiracles and the animals allowed to recover. The mean total rate of waterloss of the animals that survived is also shown in Table III.1. It is notable that the rate of waterloss in these animals ( $2.45 \text{ mgm gm}^{-1} \text{ hr}^{-1}$ ) is little different from that of the control animals ( $2.5 \text{ mgm gm}^{-1} \text{ hr}^{-1}$ ).

Table III.1 Effect of blockage of the booklungs of dead and live *P. antipodiana* on the total rate of waterloss. Booklung openings blocked with nail varnish. A small amount of nail varnish was applied to the abdominal cuticle of live control animals.

	Rate of waterloss ( $\text{mgm gm}^{-1} \text{ hr}^{-1}$ )
Live controls (n = 10)	$2.5 \pm 0.3$
Live animals booklungs blocked (n = 5)	$2.45 \pm 0.5$
Dead animals booklungs blocked (n = 10)	$6.3 \pm 0.6^{***}$

\*\*\* Rate of waterloss of dead animals significantly higher than control animals ( $p < 0.001$ ), Student t test.

Among the insects, closer mechanisms associated with the spiracular openings of the trachea limit waterloss from the respiratory surfaces (Mellanby, 1934; Bursell, 1959; Miller, 1964a, b; Loveridge, 1968b). Valves covering the spiracular openings of the booklungs of spiders have been described (Millot, 1949; Robinson and Paim, 1968) and it is thought that they may function to limit waterloss from the booklungs (Davies and Edney, 1952; Cloudsley-Thompson, 1957).

The booklungs and spiracles of *P. antipodiana* have not previously been described. They conform to the general arrangement seen in mygalomorph spiders and structurally resemble those described for the araneamorphs (Robinson and Paim, 1969). Two pairs of booklungs and spiracles are located in the ventral anterior region of the abdomen.

Externally the position of the booklungs is easily distinguished by the pale sclerotised patches which form the ventral wall of the booklung atria.

Each booklung is a pouch formed by the invagination of the integument (Kastner, 1924). The anterior region of the pouch is filled with lung lamellae and the posterior portion consists of an atrium which opens to the exterior via a slit, the booklung spiracle. A rigid fold of the body wall, which continues as the ventral wall of the atrium, forms the anterior edge of the spiracle. The posterior edge of the spiracle consists of a flexible fold of the ventral wall of the abdomen. This fold functions as the spiracular valve folding inwards and backwards.

Ventilation of the booklungs of *P. antipodiana* was not observed and it would appear that, as in spiders in general, diffusion suffices for respiratory exchange (Meglistch, 1972).

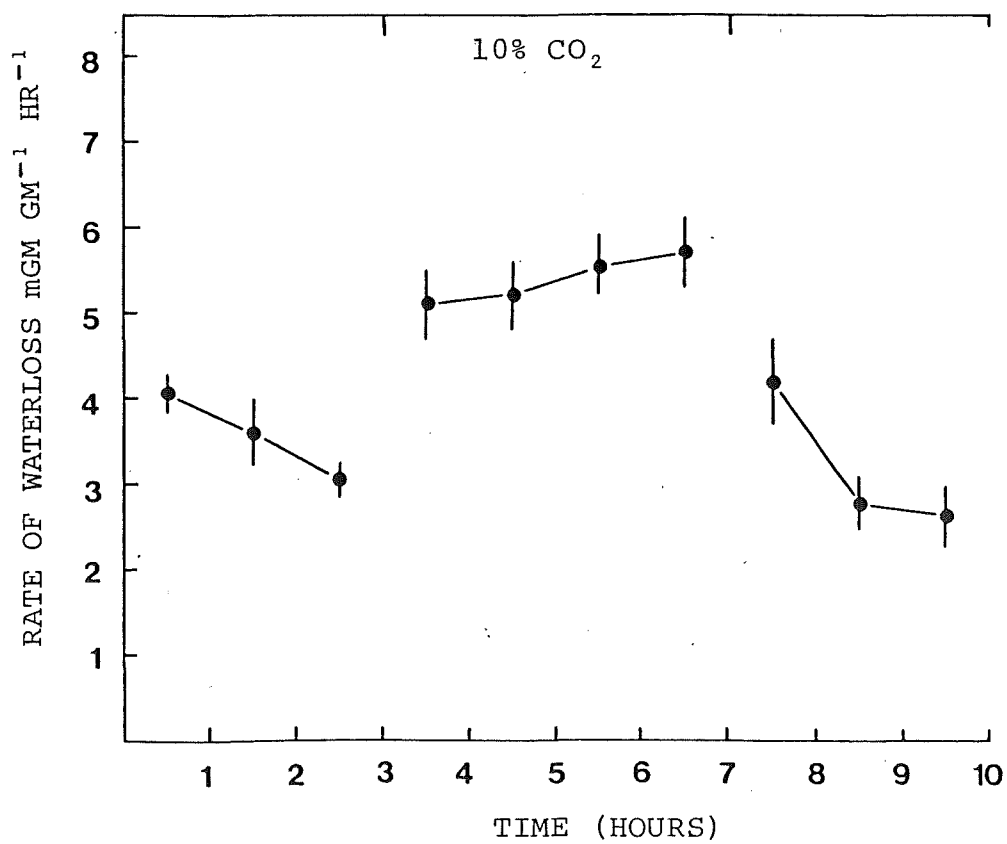
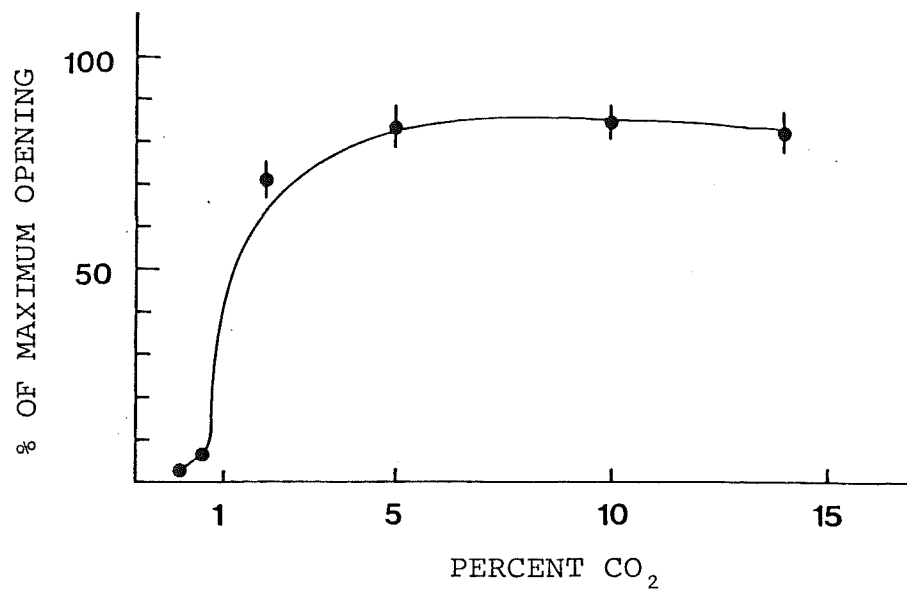
The extent of opening of the spiracular valve could be controlled with air/CO<sub>2</sub> mixtures. In normal air, the spiracular valve of the resting animal fluttered rapidly and irregularly over a range of 0 to 2.5% of maximum opening. The speed of the movements was such that it was not possible to follow them with the ocular micrometer. Activity of the spider, such as struggling against the wax bonds, resulted in an increased opening of the spiracular valve to 30 - 50% of maximum. The opening of the valve coincided with the onset of activity and, following brief periods of activity, the valve returned to the resting position immediately the activity ceased. During sustained activity, induced by prodding the chelicerae of the animal with a paint brush, the spiracular valves remained open continuously and remained open for varying periods once activity ceased.

Exposure of the spiders to CO<sub>2</sub> caused the spiracular valves to open, the extent of opening increasing with the concentration of CO<sub>2</sub> (Figure III.5). In 2% CO<sub>2</sub> the spiracular valve was 71% open, increasing to 84% in 5% CO<sub>2</sub>. Exposure to 10% and 15% CO<sub>2</sub> did not increase the measured extent of opening, however, the magnitude of the response in 2% and 5% CO<sub>2</sub> decreased with the duration of exposure, whereas in 10% and 15% CO<sub>2</sub> no measurable reduction in response occurred, even after 4 hours. Following exposure to 10% and 15% CO<sub>2</sub> there was a delay in the return of the spiracular valve to the normal position.



Figure III.5 The extent of opening of the spiracular valves of *P. antipodiana* in different concentrations of CO<sub>2</sub>. Opening expressed as a percentage of maximum opening, which was measured in 100% CO<sub>2</sub>. Vertical lines =  $\pm 1$  S.E.M., n = 8.

Figure III.6 The effect of sustained opening of the spiracular valves in dry air on the total waterloss of *P. antipodiana*. Vertical lines =  $\pm 1$  S.E.M., n = 8.



### III.3.3 The Effect of Sustained Opening of the Spiracular Valves on Total Waterloss

Ten percent CO<sub>2</sub> caused the spiracular valves of *P. antipodiana* to remain 85% open. The effect of this sustained opening of the spiracular valves on the total waterloss of *P. antipodiana* is shown in Figure III.6. Spiders, pre-treated as described in the methods, were exposed to normal dry air for three hours, a dry gas mix of 90% air 10% CO<sub>2</sub>, for four hours then returned to normal dry air. During the initial three hour exposure to dry air, the rate of waterloss gradually declined from 4.0 to 3.05 mgm gm<sup>-1</sup> hr<sup>-1</sup>. Exposure to 10% CO<sub>2</sub> in the fourth hour resulted in a 65% increase in total waterloss, and this elevated rate was sustained, with a slight increase, for the full four hour exposure to 10% CO<sub>2</sub>. The return to normal air resulted in a decrease in the rate of waterloss, but it was two hours before waterloss returned to levels measured before exposure to 10% CO<sub>2</sub>. This is consistent with the delay in closure of the spiracular valves observed following exposure of spiders to 10% and 15% CO<sub>2</sub>.

### III.3.4 Waterloss Associated with Silk Production

There are two possible sources of water loss associated with the construction of a web:

- (i) Increased respiratory waterloss resulting from increased activity associated with web construction.
- (ii) Loss of water directly from the silk as it is drawn from the spinnerets.

It was noted that web construction always occurred when the spiders were placed in clean containers, the period of activity involved in constructing the web lasting as long as 45 minutes. Therefore, to differentiate between the two possible sources of water loss associated with the web the following experiment was carried out during web construction. Two groups of spiders were employed, one in which the spinnerets had been blocked with nail polish, the second in which a small drop of nail polish had been applied to the abdominal cuticle. Each group was placed in clean containers and the rate of waterloss measured during web construction by the control spiders and for an equivalent period by the spiders with their spinnerets blocked. Web constructing behaviour

was obvious, the spiders moved around the edges of the container applying the spinnerets to the walls and floor of the container. Animals with blocked spinnerets initiated and sustained the behaviour as readily as the control animals.

The mean rates of waterloss of the two "spinning" groups are shown in Table III.2. Also shown is the rate of waterloss of non-spinning normal spiders (from III.3.2).

Table III.2 The effect of blockage of the spinnerets on the rate of waterloss of *P. antipodiana* during web construction. Spinnerets blocked with nail polish. Control spiders have small drop of nail polish on the abdominal cuticle. Non-spinning spiders inactive in the web ( $\bar{X} \pm \text{S.E.M.}$ ,  $n = 10$ ).

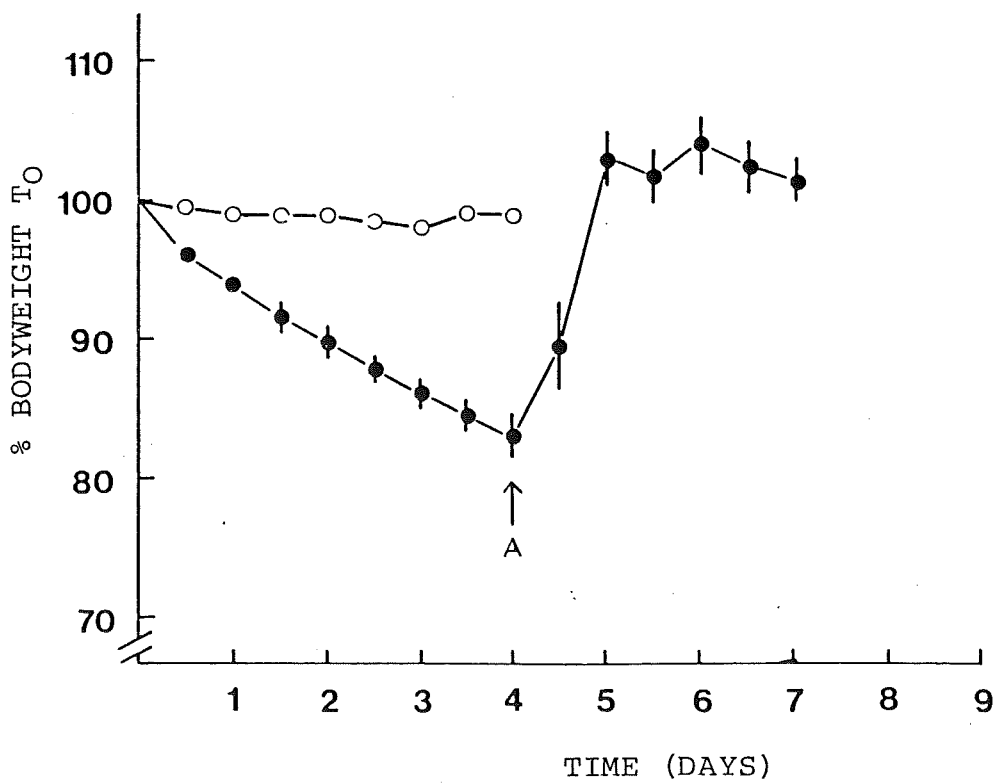
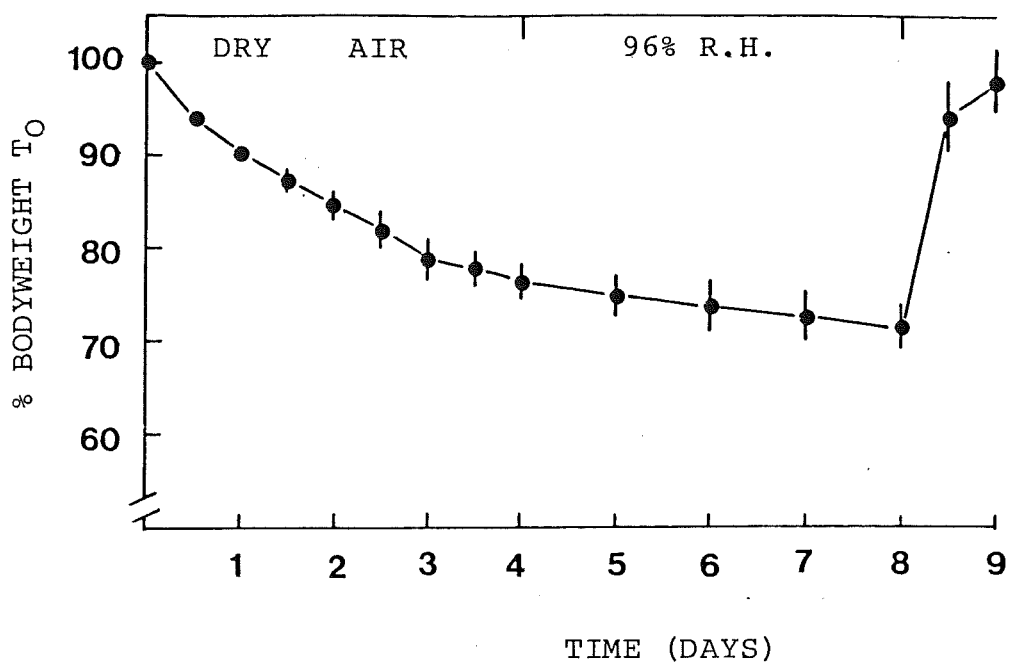
	Rate of waterloss (mgm gm <sup>-1</sup> hr <sup>-1</sup> )
Non-spinning controls	2.5 $\pm$ 0.3
Spinning controls	3.2 $\pm$ 0.15
Blocked spinnerets	3.4 $\pm$ 0.20*

\* Waterloss significantly higher than non-spinning control,  $p < 0.05$ , Student t test.

The rate of water loss in the two "spinning" groups was similar and both were elevated when compared to non-spinning controls. The difference may, however, be due to the fact that waterloss in the non-spinning group was measured over three hours while the spinning spiders only maintained spinning behaviour for 45 - 60 minutes. Although all three groups involved were maintained at room temperature and humidity for five hours to ensure the removal of water loosely associated with the cuticle, it has been shown above (III.3.3) that waterloss decreased with time even after the removal of this water. The results do indicate, however, that waterloss directly associated with the web is small compared to the total waterloss.

Figure III.7     The continued loss of water by *P. antipodiana* in 96.5% R.H. following four days of desiccation in dry air at 20°C.     Vertical lines =  $\pm 1$  S.E.M.,  $n = 8$ .

Figure III.8     Time course of changes in bodyweight during dehydration of *P. antipodiana*.     Open symbols, control animals provided with water,  $n = 8$ .     Closed symbols, experimental dehydrated in dry air at 20°C then provided with water at A,  $n = 8$ .     Vertical lines =  $\pm 1$  S.E.M.



### III.3.5 Absorption of Water from the Atmosphere

Unlike a number of insects (Beament *et al.*, 1964; Edney, 1966; Knulle, 1967; Knulle and Spadofora, 1969; Machin, 1975) and acarines (Sauer and Hair, 1971; Hair *et al.*, 1975) *P. antipodiana* cannot gain weight by the reabsorption of water from the atmosphere. Shown in Figure III.7 are changes in the bodyweight of spiders which were dehydrated at 0% R.H. for four days then transferred to 96.5% R.H. In the elevated humidity the spiders continued to lose weight, although at a much reduced rate ( $0.23 \text{ mg gm}^{-1}\text{hr}^{-1}$ ) compared to their mean rate of waterloss in 0% R.H. ( $1.86 \text{ mg gm}^{-1}\text{hr}^{-1}$ ). Following exposure to 96.5% R.H. the spiders were provided with free water and they gained most of their lost weight within 24 hours.

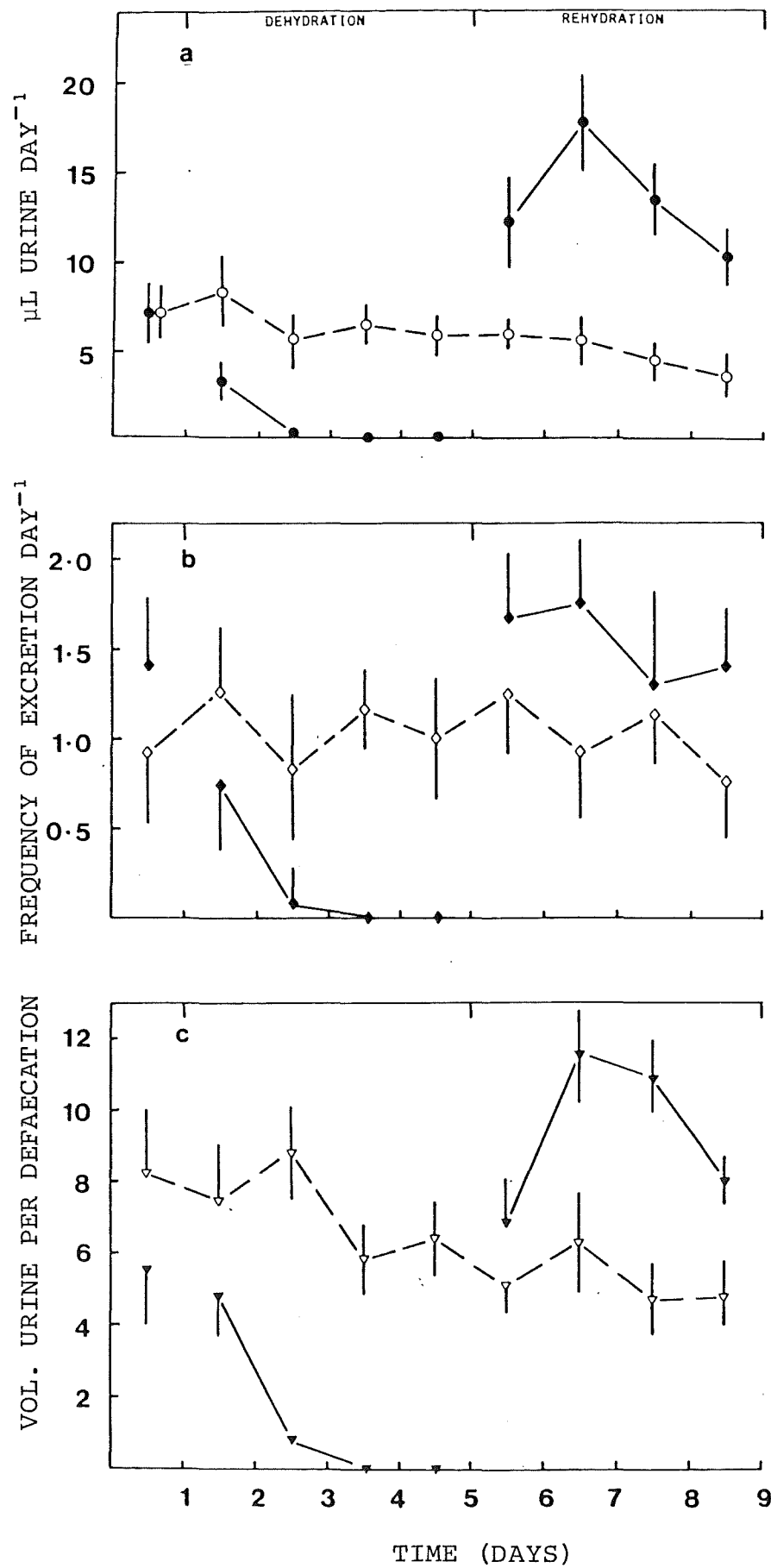
### III.3.6 The Effects of Dehydration and Rehydration

The degree to which the haemolymph osmotic pressure increases during dehydration varies among the terrestrial arthropods. The terrestrial crustaceans show little regulation of their haemolymph composition during dehydration (Bliss, 1968; Greenaway and MacMillen, 1978; Harris and Kormanik, 1981), and so increases in the haemolymph osmotic pressure agree with what is predicted from measured waterlosses. The insects, however, show varying degrees of regulation (Edney, 1968; Hyatt and Marshall, 1978) and often very small increases in haemolymph composition occur in spite of large reductions in the haemolymph volume through dehydration.

The effects of dehydration and rehydration on the haemolymph composition of *P. antipodiana* were measured in the following experiment. Sixteen hydrated fed *P. antipodiana* (weight range 0.8540 - 0.9810 gm) were taken from the culture and starved for two days before being dehydrated at 0% R.H. After four days dehydration, haemolymph samples were collected from half of the animals while the remainder were provided with free water and allowed to rehydrate. After two days rehydration, haemolymph samples were collected from the remaining animals. The composition of the haemolymph of the two experimental groups was compared to that of hydrated controls, which were starved for six days after removal from the culture. Similar experiments with different groups of spiders were carried out to measure the effects of dehydration and

Figure III.9 The effect of dehydration and rehydration on the excretion of urine by starved *P. antipodiana*. (a) Rate of urine excretion. (b) Frequency of excretion. (c) Volume of urine per defaecation. Vertical lines =  $\pm 1$  S.E.M. Open symbols, control spiders provided with water,  $n = 10$ . Closed symbols, experimental spiders, dehydrated at 0% R.H. and 20°C then rehydrated with water,  $n = 10$ .





rehydration on the bodyweight, anal urine production and the excretion of Na and K via the anus. Coxal excretion was not measured. It should be noted, however, that although the coxal glands do not excrete during periods of dehydration and starvation, they do excrete fluid during rehydration following water deprivation (Section IV).

### III.3.6 (a) Changes in weight

Shown in Figure III.8 are the changes in the mean bodyweight of eight spiders dehydrated for four days then provided with water and allowed to rehydrate. Dehydration resulted in a 16% loss of bodyweight, however, within one day of being provided with free water all of the lost weight was regained. In animals rehydrated with an 8 mM amaranth solution, rather than water, the contents of the foregut, midgut diverticula and stercoral pocket were all stained bright red, suggesting that the weight was regained by drinking. This does not, however, exclude the possible contribution of water absorption mechanisms such as the extensible vesicles employed by the collembolan *Onychiurus armatus* (Nutman, 1941), the diplurid *Campodea* (Drummond, 1953), and the symphylid *Hanseniella* (Tiegs, 1947). However, observations of rehydrating spiders did not indicate the presence of similar structures in *P. antipodiana*.

### III.3.6 (b) Anal excretion during dehydration and rehydration

During the period of dehydration the rate of urine excretion decreased as described above (Section III.3.1). The reduction resulted from a decrease in both the frequency of excretion and the volume of urine excreted per defaecation (Figure III.9). Interestingly, during rehydration *P. antipodiana* experienced a period of diuresis during which the rate of excretion of the rehydrating animals rose to a maximum level of  $17.8 \mu\text{l day}^{-1}$ , more than three times the rate of control animals in the same period. During the diuresis there was a small increase in the frequency of excretion and a large increase in the volume of urine excreted per defaecation (Figure III.9).

Accompanying the decrease in urine excretion during dehydration was a decrease in the rate of Na and K excretion via the anus (Figure III.10). Both Na and K excretion increased again during the diuresis associated with rehydration. However, it is interesting to note that

Figure III.10    Effect of dehydration and rehydration on the excretion of Na and K in the excreta of starved *P. antipodiana*.  
(a) Rate of Na excretion.    (b) Rate of K excretion.  
(c) Na/K ratio of the excreta.    Vertical lines =  $\pm 1$  S.E.M.  
Open symbols, control spiders provided with water, n = 10.  
Closed symbols, experimental spiders, dehydrated at 0% R.H. and 20°C then rehydrated with water, n = 10.

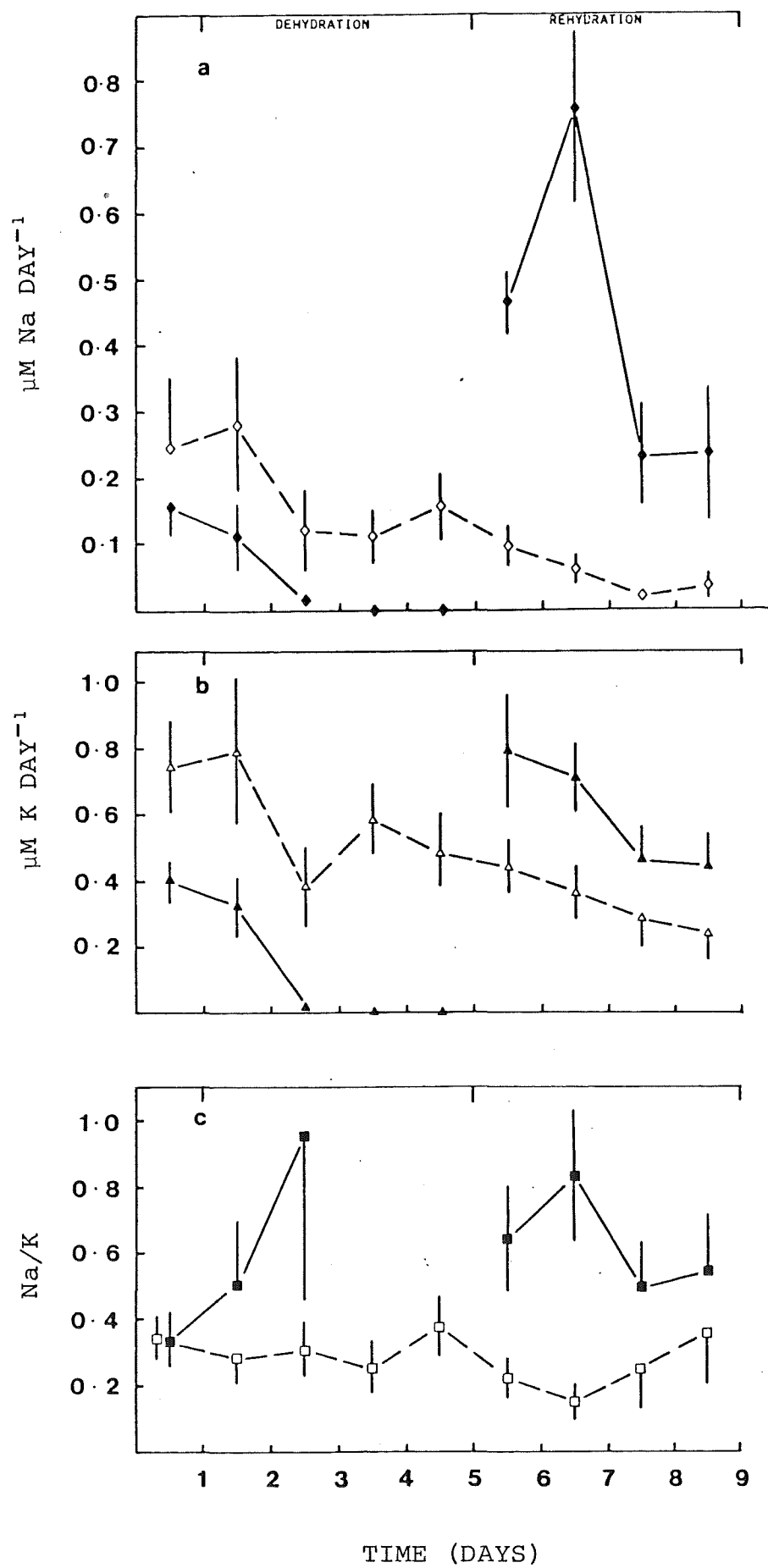


Table III.3 The effect of dehydration and rehydration on the haemolymph composition of *P. antipodiana*.  
Hydrated control animals starved for four days. All values  $\bar{X} \pm \text{S.E.M.}$

	Hydrated Starved n=8	Dehydrated 96 hours n=8	Dehydrated 96 hours Rehydrated 48 hours n=8
Osmotic pressure mOsm (kg H <sub>2</sub> O) <sup>-1</sup>	432 $\pm$ 11.6	631 $\pm$ 24***	452 $\pm$ 9
Na mM l <sup>-1</sup>	228 $\pm$ 7.6	313 $\pm$ 7.3***	201 $\pm$ 8.3*
K mM l <sup>-1</sup>	5.2 $\pm$ 0.6	8.3 $\pm$ 0.7**	4.8 $\pm$ 0.9
Cl mM l <sup>-1</sup>	180.6 $\pm$ 5.6	253 $\pm$ 7.6***	159 $\pm$ 14

Mean values for dehydrated and dehydrated/rehydrated animals compared to mean values for hydrated controls by unpaired Student t test.

Significance levels of difference between means: \*, 0.05 > p > 0.01; \*\*, 0.01 > p > 0.001; \*\*\*, 0.001 > p.

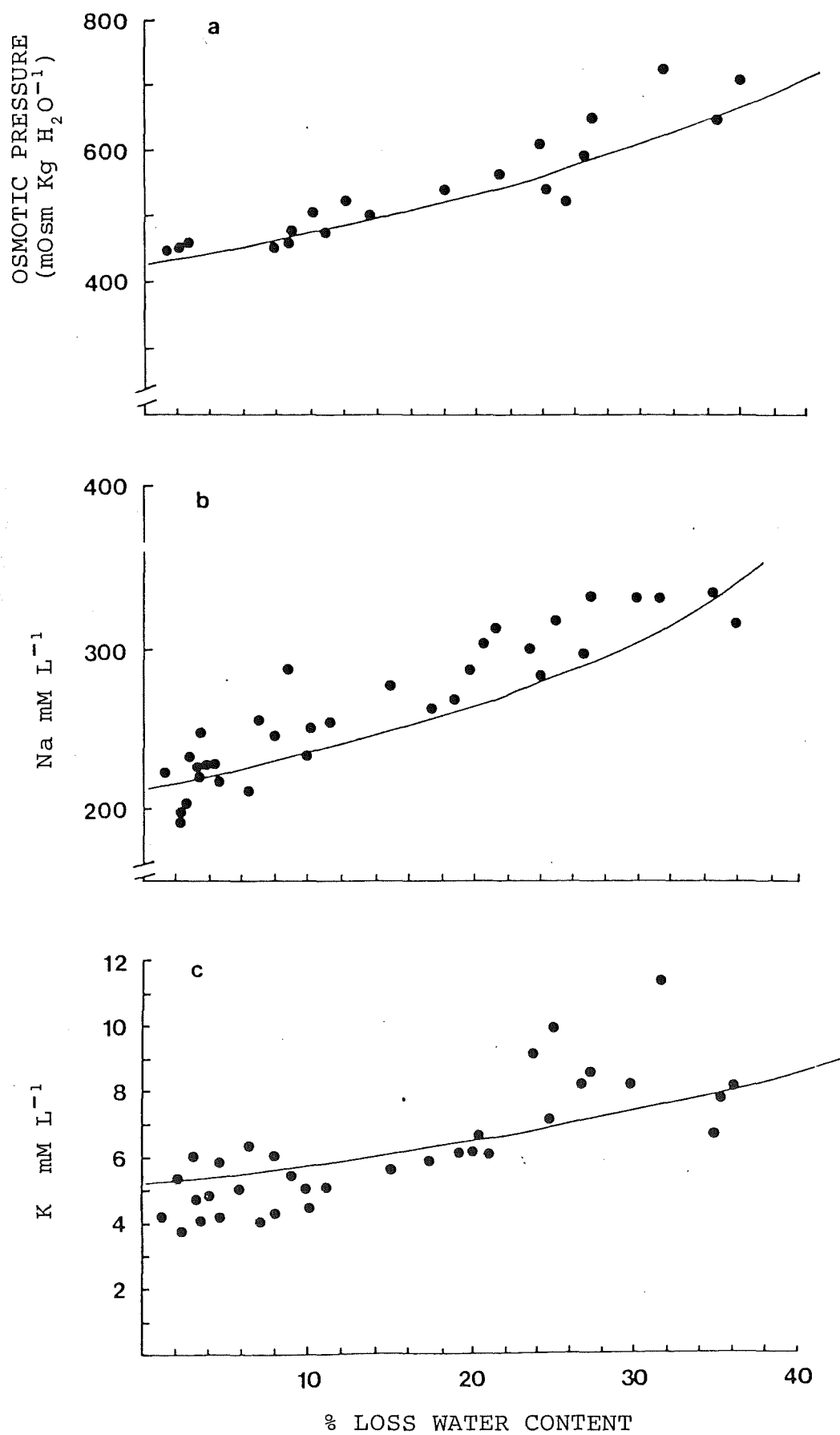
the increase in Na excretion during this period is far greater than that of K. Rehydrating animals excreted Na at a rate up to seven times that observed for controls, whereas maximum K excretion during rehydration was only 1.8 times that of the controls.

### III.3.6 (c) The effect of dehydration and rehydration on the haemolymph composition

A summary of the composition of the haemolymph of hydrated, dehydrated and dehydrated/rehydrated animals is given in Table III.3. Four days dehydration at 0% R.H. resulted in a 46% increase in the haemolymph osmotic pressure, 37% increase in Na concentration, 60% increase in K concentration, and a 40% increase in Cl concentration. Upon rehydration the haemolymph osmotic pressure returned to levels slightly above that of the controls while the haemolymph Na, K and Cl concentrations were less than control values.

It would appear that there is little regulation of the haemolymph composition of *P. antipodiana* during dehydration. In Figure III.11 the osmotic pressure, Na and K concentrations of the haemolymph of spiders dehydrated for varying periods of time at 0% R.H. and 20°C are plotted against the percentage waterloss of the spiders. The solid lines represent the theoretical increases that would occur in the haemolymph concentrations assuming that water was lost at proportionately similar rates from the haemolymph and the body tissues, and that no net movements of ions or osmotically active substances occurred between the two compartments. The rise in the osmotic pressure, Na and K concentrations observed conform approximately to the predicted increases. It is therefore probable that the assumptions made in the prediction pertain, and that little regulation of the haemolymph composition occurs during dehydration. There are alternative explanations, and although they are unlikely, they cannot be ruled out. Thus preferential water retention by or loss from the haemolymph compartment in association with a net influx or outflux of electrolytes could, under certain circumstances, explain the results.

Figure III.11    Effect of dehydration on the osmotic pressure and Na and K concentration of the haemolymph of *P. antipodiana*. The solid lines indicate the expected concentrations calculated on the assumptions that a given percent loss of body water causes a similar percent loss of haemolymph volume and no net movement of ions into or out of the haemolymph occurs.    (a) osmotic pressure.    (b) Na concentration.    (c) K concentration.





### III.4 DISCUSSION

In Section I it was shown that the major avenue of waterloss from *P. antipodiana* consisted of transpirational losses across the cuticle and the respiratory surfaces. Also included in the measure of transpirational loss in Section I were losses associated with the web. The results presented here indicate that of those losses, cuticular loss was the most important. Losses associated with respiration at rest and the silk were insignificant with regard to this cuticular component. On the other hand, activity associated with web construction increased the total waterloss markedly, and urinary excretion contributed significantly to total loss but during periods of dehydration the rate of urine excretion decreased rapidly to zero.

Interestingly, unlike many terrestrial insects *P. antipodiana* exhibited little ability to regulate its haemolymph composition during dehydration.

In spite of the relative importance of cuticular waterloss to *P. antipodiana* it should be noted that the cuticular permeability of *P. antipodiana* is comparable with that of other terrestrial arthropods. In Table III.4 the rate of waterloss of *P. antipodiana*, expressed as  $\mu\text{g cm}^{-2} \text{ hr}^{-1} (\text{mm Hg saturation deficit})^{-1}$ , is compared with rates of waterloss of other terrestrial arthropods. Expression of waterloss in this form gives a measure of the permeability of the cuticle to water (Edney, 1977), and facilitates comparisons between animals of different sizes. The cuticular permeability of *P. antipodiana* ( $9.0 \mu\text{g cm}^{-2} \text{ hr}^{-1} \text{ mm Hg S.D.}^{-1}$ ) was low enough to rank *P. antipodiana* among animals inhabiting xeric environments. In all classes of arthropods there are good examples of a close relationship between the permeability of the animals and their habitat (Norgaard, 1951; Davies and Edney, 1952; Edney, 1961; Duffey, 1962; Herreid, 1969a; Almquist, 1971; Edney, 1977), thus the low cuticular permeability of *P. antipodiana* is perhaps unexpected when the habitat from which the spiders were collected is considered. *P. antipodiana* is generally found in moist, hygric conditions, such as under rocks in damp ground, and fallen trees. At Whalers Bay, Kaikoura, *P. antipodiana* was collected from beneath rocks which are stacked among the grass in the supra-littoral zone. The spiders spin funnel-like webs which extend down among the rocks and shingle, often to a depth of 40 cm, and the habitat at its driest would

Table III.4 Selective data on the permeability of the cuticle of arthropods from different habitats. Permeability measured at temperatures between 20° and 30°C.

\* The permeability of the cuticle of *P. antipodiana* was calculated from transpirational loss only, i.e. total loss minus loss due to defaecation.

Taxon	Habitat	Permeability $\frac{\mu\text{g cm}^{-2}\text{hr}^{-1}}{\text{mm Hg}^{-1}}$	Reference
Decapod crustaceans			
<i>Uca annulipes</i>	hygic	80	Edney (1961)
<i>U. marionis</i>	hygic	200	Edney (1961)
Isopod crustaceans			
<i>Porcellio scaber</i>	hygic	110	Edney (1951)
<i>Hemelepistus reaumuri</i>	xeric	23	Cloudsley-Thompson (1956)
<i>Venezillo arizonicus</i>	xeric	32	Warburg (1965)
Insects			
<i>Hepialus</i> larvae	hygic	190	Wigglesworth (1945)
<i>Nematus</i> larvae	hygic	20	Wigglesworth (1945)
<i>Blatta orientalis</i>	mesic	48	Mead-Briggs (1956)
<i>Calliphora erythrocephala</i>	mesic	51	Mead-Briggs (1956)
<i>Periplaneta americana</i>	mesic	55	Mead-Briggs (1956)
<i>Glossina morsitans</i>	mesic - xeric	8	Bursell (1957a)
<i>Areniraga investigata</i>	xeric	12.1	Edney & McFarlane (1974)
<i>Centrioptera muricata</i>	xeric	6.3	Ahearn (1970)
<i>Cryptoglossa verrucosa</i>	xeric	8.4	Ahearn & Hadley (1969)
<i>Eleodes armata</i>	xeric	17.2	Ahearn & Hadley (1969)
<i>Eremiaphila monodi</i>	xeric	7.0	Delye (1969)
<i>Locusta migratoria</i>	xeric	22	Loveridge (1968b)
<i>Rhodnius prolixus</i>	xeric	12.0	Holdgate & Seal (1956)
<i>Tenebrio molitor</i> (larvae)	xeric	5	Mead-Briggs (1956)
<i>Thermobia domestica</i>	xeric	15	Beament (1964)
<i>Chortoicetes terminifera</i>	mesic	41	Lees (1976)
Myriapods			
<i>Glomeris marginata</i>	hygic	200	Edney (1951b)
<i>Lithobius</i> sp.	hygic	270	Mead-Briggs (1956)
<i>Orthoporus ornatus</i>	xeric	7.9	Crawford (1972)

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Arachnids

## Ticks

<i>Ixodes ricinus</i>	mesic	60	Lees (1947)
<i>Ornithodoros moubata</i>	xeric	4	Lees (1947)
<i>O. savignii</i> (♀ unfed)	xeric	2	Hafez <i>et al.</i> (1970)
<i>Hyalloma dromedarii</i> (♀ unfed)	xeric	12	Hafez <i>et al.</i>

## Scorpions

<i>Pandinus imperator</i>	mesic	76	Cloudsley-Thompson (1959)
<i>Androctonus australis</i>			
<i>Androctonus australis</i>	xeric	0.8	Cloudsley-Thompson (1956)
<i>Buthotus minax</i>	xeric	0.98	Cloudsley-Thompson (1962)
<i>Hadrurus arizonensis</i>	xeric	1.22	Hadley (1970)
<i>H. hirsutus</i>	xeric	25	Cloudsley-Thompson (1967)
<i>Leirurus quinquestriatus</i>	xeric	1.27	Cloudsley-Thompson (1961)

## Spiders

<i>Lycosa amentata</i>	mesic	28.3	Davies & Edney (1952)
<i>Eurypelma</i> sp.	xeric	10.3	Cloudsley-Thompson (1967)
<i>Dugesiiella hentzi</i>	xeric	10	Stewart & Martin (1970)
<i>Ciniflo similis</i>	mesic	11.1	Cloudsley-Thompson (1957)
<i>C. ferox</i>	mesic - hygric	18.2	Cloudsley-Thompson (1957)
<i>C. fenestralis</i>	mesic - hygric	21.1	Cloudsley-Thompson (1957)
<i>Porrhothele antipodiana</i> *	mesic - hygric	9	This study

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be mesic. Variations in the availability of water in this habitat were noted, and there appeared to be behavioural differences associated with these variations. During autumn, winter and early spring considerable moisture from rainfall and dew was present on the webs of *P. antipodiana*, and the spiders were usually collected from the upper regions of the webs. During late spring and summer, however, the habitat often became dry and a high moisture content in the web was only noted at dawn. During this period spiders were only collected from the upper region of the web at dawn and dusk, during the rest of the day the spiders were found deep within their webs.

Interestingly, the permeability of the cuticle of *P. antipodiana* also appeared to vary with the season. Rates of waterloss presented here were measured in animals collected in mid-summer (1978). Animals collected in the winter (1979) had higher cuticular permeabilities,  $34.8 \pm 3 \mu\text{g cm}^{-2} \text{ hr}^{-1} \text{ mm Hg S.D.}^{-1}$ . Hadley (1978) and Toolson and Hadley (1979) have shown that the lipid composition of the cuticle of insects and arachnids varies with the season and, furthermore, in the scorpion, *Centruroides sculpturatus*, it was shown that the variations in cuticular lipid composition could be correlated with variations in the permeability of the cuticle, summer scorpions having a permeability as low as 26% of that of winter animals (Hadley, 1979).

The rate of waterloss of *P. antipodiana* decreases with time, even when allowance is made for the reduction in urine production (Figure III.3). Part of the reduction could result from a lower level of activity from reduced web spinning. It was noted in hydrated animals that although the web was permanent, silk was added to the web each day. The webs of severely dehydrated animals, however, were often in a state of disrepair and dehydrated animals transferred to clean containers often did not construct webs. Increased control of respiratory waterloss could also contribute to the observed reduction in total transpiration. However, respiratory loss of *P. antipodiana* at 20°C and in dry air is a very small component of the total transpirational loss, thus little further reduction could be achieved in this manner. Therefore, it would appear that the observed reduction in the transpirational loss of *P. antipodiana* was due to a reduction in the permeability of the cuticle. Reductions in cuticular permeability with dehydration have been reported for a number of other terrestrial arthropods; isopods (Edney, 1951; Bursell, 1955); land crabs (Herreid, 1969a,b); myriapods (Perttunen, 1953; Crawford, 1972); scorpions

(Cloudsley-Thompson, 1956, 1967; Hadley, 1970; Crawford and Wooten, 1973); spiders (Humphreys, 1975) and insects (Schmidt, 1955; Loveridge, 1968a; Edney, 1971).

Bursell (1955) suggested that the reductions in the waterloss of the terrestrial isopod *Oniscus ocellus* resulted from the increased haemolymph electrolyte concentrations, that occur during dehydration of this animal, affecting protein constituents of the cuticle in such a way as to cause contraction, leading to an increased resistance to water. In view of the increased haemolymph osmotic pressure that occurs in *P. antipodiana* during dehydration, a similar mechanism may be responsible for the reduction in cuticular permeability observed in *P. antipodiana*. Alternatively, the large differences in the rates of waterloss of living and dead spiders suggest that the epidermal cells beneath the cuticle may be a significant permeability barrier (Berridge, 1970) in *P. antipodiana*, and variations in the permeability of the epidermal cells, perhaps mediated hormonally, may explain the reductions in the permeability of the cuticle. Treherne and Willmer (1975) demonstrated that the permeability of the cuticle of the cockroach *Periplaneta americana* appeared to be under hormonal control, the rate of waterloss of live decapitated animals exceeding that of controls.

The small contribution of respiratory waterloss to the total in resting *P. antipodiana* was not unexpected. Davies and Edney (1952) reported that at 20°C respiratory waterloss of the spider *Lycosa amentata* was an insignificant component of the total transpirational loss, although with increased temperature and metabolic rate its contribution increased. Spiders are recognised as having very low metabolic rates compared with other arthropods of similar size (Dresco-Derouet, 1960, 1971, 1973; Miyashata, 1969; Anderson, 1970; Seymour and Vinegar, 1973; Greenstone and Bennett, 1980; McQueen, 1980). Therefore, assuming some form of spiracular control a low rate of respiratory waterloss would be expected.

Spiracular valves have been described in a number of spiders (Kastner, 1929; Millot, 1949; Robinson and Paim, 1969; More, 1976) and it has been demonstrated that CO<sub>2</sub> and other gases affect the extent of opening of the valves (Hazelhoff, 1926; Robinson and Paim, 1969). A number of authors have reported the effects of CO<sub>2</sub> on the total waterloss and reported similar increases as were observed here. *Lycosa amentata* showed a 43% increase of total waterloss in a 10% CO<sub>2</sub>/dry air mixture (Davies and Edney, 1952). The

rate of waterloss of the tarantula, *Eurypelma hentzi*, increased 76% in 15% CO<sub>2</sub>/dry air (Stewart and Martin, 1970) and Cloudsley-Thompson (1957) reported increases in the rate of waterloss in 10% CO<sub>2</sub>/dry air for the spiders *Ciniflo ferox*, *C. similis* and *C. fenestralis* of 67%, 63% and 86% respectively.

Robinson and Paim (1969) investigated the response of the spiracular valves of *Araneus diadematus* and *A. marmoreus* to various gas mixtures and likened their operation to the spiracular valves of larval insects (Buck, 1962), which open periodically to release short bursts of CO<sub>2</sub>. In *P. antipodiana*, however, the spiracular valves were always at least partially open (2 - 5% of maximum) at rest and further opening was always associated with increased activity. It would appear that the spiracular valves of *P. antipodiana* operate in a manner similar to that reported for a number of adult insects, restricting the extent of opening of the spiracles during periods of low metabolic activity and so limiting water loss (Bursell, 1957, 1974; Miller, 1964).

Although the information reported here and by other authors confirms that the spiracular valves can limit waterloss in the spiders, it remains to be demonstrated whether variations in the extent of control by the valves occurs with changes in the hydration state of the spiders, as has been shown for insects (Bursell, 1957; Miller, 1964; Krasfur, 1971b). Certainly in *P. antipodiana* at temperatures at or below 20°C little further control could be achieved in the resting animal, however, in insects increased levels of activity or CO<sub>2</sub> are required to open the spiracles of dehydrated animals (Miller, 1964). Similar increases in the threshold of the opening response would reduce respiratory waterloss during activity of *P. antipodiana*.

Activity associated with web construction did increase the rate of waterloss of *P. antipodiana*. A result which would be predicted from observations of the spiracular valves of struggling spiders. Interestingly, the increase in waterloss due to activity associated with web construction greatly exceeded any possible waterloss from the web itself as there was no difference between the rates of waterloss during web construction of spiders with their spinnerets blocked or unblocked, although both showed similar increases due to activity. Peakall (1969) noted that the duct of the ampullate gland of *Araneus sericatus* is some five times longer than would be necessary if its sole function was to connect the main part of

the gland to the spinnerets. Furthermore, electron microscopy of the duct (Bell and Peakall, 1969) revealed a morphological structure similar to that associated with water reabsorbing structures, and it has been demonstrated that the water content of the silk is reduced markedly after its passage through the duct (Witt *et al.*, 1968).

In the long term the investment in terms of water made by the spider during the construction of the web should be more than recouped. The web increases the chances of the spider capturing prey, a source, albeit limited, of water to the spider (Sections I and IV). Furthermore, droplets of water condensed on the web of *P. antipodiana* were often observed in the field. *P. antipodiana* readily drinks free water and this may be an important source of water under natural conditions. Finally, the web constructed by *P. antipodiana* is a thick funnel-like structure and the spider adopts a stationary position in the narrow portion of the web where it is closely surrounded by silk. The web may alter the micro-environment of the spider increasing the local relative humidity and thus reducing waterloss. Often in the laboratory *P. antipodiana* would spin a flat, sheet-like web rather than a funnel. Rates of waterloss of these spiders over 24 hours were higher than those observed for animals in normal webs (Table III.5). Although it should be noted that no measure of activity was made of the animals and so differences may be due to increased activity.

Table III.5      Rate of waterloss of spiders in normal funnel-like webs and spiders on sheet-like webs.    ( $\bar{X} \pm \text{S.E.M.}$ )

	Rate of Waterloss ( $\text{mgm gm}^{-1} \text{ hr}^{-1}$ )
Normal web (n = 30)	1.9 $\pm$ 0.10
Sheet web (n = 10)	3.19 $\pm$ 0.22***

\*\*\* Significant difference between means,  $p < 0.001$ , Student  $t$  test.

Like the terrestrial crustaceans (Bliss, 1968; Greenaway and MacMillen, 1978; Harris and Kormanik, 1981), and unlike many terrestrial insects, *P. antipodiana* does not regulate its haemolymph composition

during dehydration; observed increases in the haemolymph osmotic pressure, Na, K and Cl concentrations agreeing with values predicted from measurements of water loss. Hadley (1974) suggested that the desert scorpion, *Hadrurus arizonensis*, did not regulate its haemolymph composition during dehydration, it simply tolerated any increases that may occur, and it has subsequently been demonstrated that several other scorpions, from both mesic and xeric environments, do not regulate their haemolymph composition during dehydration (Riddle *et al.*, 1976; Warburg *et al.*, 1979).

The absence of haemolymph regulation by *P. antipodiana* and scorpions is, perhaps, not surprising. Loss of tissue water during dehydration will result in an increased tissue osmotic pressure and so water will be drawn from the haemolymph into the tissues. If the haemolymph osmotic pressure is regulated during this process, the haemolymph volume will be depleted in favour of the tissues, the haemolymph effectively acting as a buffer for the tissues. Mellanby (1939) originally proposed that one of the functions of the haemolymph of insects was to act as a tissue store from which water lost from the tissues during dehydration could be replenished, and subsequent investigations have confirmed that this is the case, proportionately greater losses of haemolymph water than tissue water occurring in a number of insects during dehydration (Nicolson *et al.*, 1974; Laird and Winston, 1975; Nicolson, 1980; Fielding and Nicolson, 1980). However, unlike the insects, spiders and scorpions employ their haemolymph for gas transport; furthermore, spiders are dependent upon the hydrostatic pressure of the haemolymph for locomotion (Parry and Brown, 1959a, b) as they lack extensor muscles in the legs (Parry, 1957). Therefore, regulation of the haemolymph composition during dehydration and the resultant depletion of the haemolymph volume that would accompany the regulation would seriously affect the efficiency of gas transport by the haemolymph and in the case of spiders restrict their locomotory ability.



## III.5 SUMMARY

1. Routes of waterloss from starved *Porrhothele antipodiana* and their control, and the effects of waterloss on the haemolymph composition of starved animals were studied. Dehydration and all measurements of waterloss were carried out at 20°C and in dry air.
2. *P. antipodiana* has a cuticular permeability ( $\mu\text{gm cm}^{-2} \text{ hr}^{-1} \text{ mm Hg}^{-1}$ ) as low as that of terrestrial arthropods from xeric environments.
3. Anal excretory waterloss of starved *P. antipodiana* rapidly declined, and ceased altogether, during dehydration.
4. Evaporative waterloss from the respiratory surfaces of resting *P. antipodiana* at 20°C and in dry air was a small component of the total loss.
5. Valves present at the openings of the booklungs of *P. antipodiana* respond to variations in atmospheric  $\text{CO}_2$  concentrations. Sustained opening of the valves, induced by elevated  $\text{CO}_2$  levels, increased total waterloss in dry air at 20°C by 65%.
6. Waterloss associated with the silk from which the web was constructed was an insignificant component of total loss. Increased activity during web construction resulted in an increase in total waterloss.
7. The haemolymph osmotic pressure, Na, K and Cl concentrations increased when spiders were dehydrated in dry air at 20°C.
8. Increases in the osmotic pressure, Na and K concentrations of the haemolymph approximated predicted increases based on the assumption that during dehydration water was lost at proportionately similar rates from the haemolymph and body tissues, and that no net movements of water and osmotically active substances occurred between the two compartments.

IV. EFFECT OF WATER DEPRIVATION AND DEHYDRATION ON THE FUNCTIONING OF THE ANAL SYSTEM AND COXAL GLANDS, AND THE SALT AND WATER BALANCE OF DEHYDRATED *PORRHOTHELE ANTIPODIANA*.

#### IV.1 INTRODUCTION

When hydrated, the spider *Porrhothele antipodiana*, employs two routes for the excretion of salts ingested with a meal, the coxal glands and the anal system. The coxal glands in hydrated animals fed prey of "normal" salt content excrete only during feeding, and produce a hypo-osmotic Na rich fluid which has been implicated in the feeding process (Section II). The anal system produces fluid at all times in the hydrated animals, and following feeding excretes a K rich fluid which is iso-osmotic to the haemolymph. However, although the fluid is iso-osmotic to the haemolymph it is markedly hypo-ionic with respect to the ions Na, K and Cl, and thus an extensive period of diuresis is required to eliminate the excess K ions ingested with the meal (Section I). Significantly the combined excretory and transpirational losses observed during the feeding and diuretic periods of hydrated *P. antipodiana* greatly exceed the water gained through the ingestion of the prey, thus hydrated *P. antipodiana* drink to maintain their water balance and support the excretion of excess salts ingested during feeding (Section I).

Perhaps the most limiting feature of the terrestrial environment is water, or more correctly, the shortage of water. The question that arises then is how do the excretory systems of water deprived dehydrated *P. antipodiana* respond to the dual, but conflicting, functions of conserving water and at the same time excreting the ions ingested with a meal?

In the terrestrial insects, excretory water losses are limited by the production of a highly concentrated urine which is often markedly hyper-osmotic to the haemolymph (Wigglesworth, 1931; Ramsay, 1952, 1955; Phillips, 1964, 1970). The ability to produce concentrated hyper-osmotic urine allows the terrestrial insects to eliminate the excess ions ingested with the meal and at the same time conserve water, and thus extract osmotically free water from the food to offset transpirational losses.

In this section the effects of water deprivation and dehydration on the functioning of the anal system and the coxal glands of *P. antipodiana* are reported. Interestingly, *P. antipodiana* is unable to produce highly concentrated excretory fluids, and thus it is limited in its ability to excrete salts during and following feeding when deprived of water or dehydrated.

The significance of this to the ion and water balance of water deprived dehydrated spiders is reported and discussed.

## IV.2 METHODS AND MATERIALS

Collection and maintenance of spiders has been described previously (Section I). All experiments were carried out at room humidity (50-70% R.H.) and  $20 \pm 2^\circ\text{C}$  on spiders which had been maintained in the laboratory for at least a week. During experiments spiders were kept in individual clean glass chambers (8 cm in diameter, 6.5 cm deep), all sides of which were lined with Whatman 542 filter paper. Each day the animals were transferred to clean containers. When required, water was provided by placing absorbent cotton wool rolls, soaked in tapwater, in small troughs on the floor of the containers. Live cockroach nymphs (*Periplaneta americana*), taken from the same culture as employed in previous sections (I and II), were used as food.

Rates of anal urine production and Na and K excretion were determined as described in Section I. The excretion of coxal fluid at times other than feeding was monitored by labelling the spiders with  $^{22}\text{Na}$ . Regions of high  $^{22}\text{Na}$  activity on the filter paper lining the experimental chambers indicate areas of dried coxal fluid (Section II). These regions were located by subdividing the filter paper into small pieces and measuring the  $^{22}\text{Na}$  activity of each piece. Areas of dry coxal fluid were transferred to appropriate volumes of 1%  $\text{HNO}_3$  and their Na and K content determined. The quantity of Na and K lost from the spiders into the prey debris through coxal excretion was determined as described in Section I.

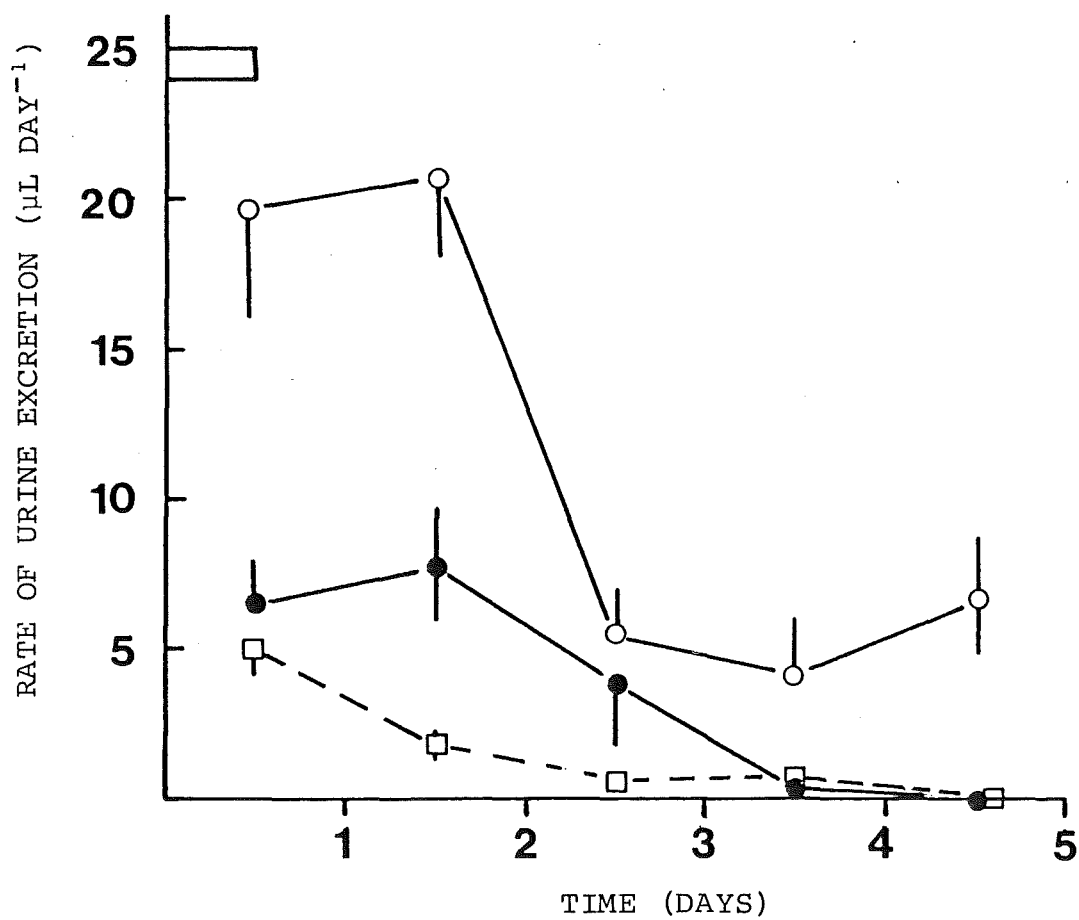
Methods of sampling haemolymph and stercoral fluid are described in Section I, as is the procedure for the injection of isotopes. Coxal fluid was collected as described in Section II, with any modifications to the protocol noted in the relevant sections. The osmotic pressure of fluid samples was measured by the cryoscopic method of Ramsay and Brown (1955), and Na and K measured with a Varian 1200 Atomic Absorption Spectrophotometer. Chloride was measured by the second method of Ramsay *et al.* (1955). The radio-isotope  $^{22}\text{Na}$  was measured with an Ortec Gamma Well Counter and  $^3\text{H}$ -inulin with a Phillips PW4540 Liquid Scintillation Counter. Sample preparation has been described in previous sections (I and II).

Figure IV.1 Effect of water deprivation during and after feeding on the rate of urine excretion of hydrated spiders fed a single meal. Vertical lines =  $\pm 1$  S.E.M. Horizontal bar represents feeding period.

○——○ Spiders fed with water available, n = 8.

●——● Spiders fed without water, n = 6.

□---□ Starved spiders deprived of water, n = 8.



### IV.3 RESULTS

#### IV.3.1 (a) The effect of water deprivation and dehydration on anal excretion following a meal

In Section I it was proposed that the large diuresis observed during and following a meal by hydrated *P. antipodiana* was supported by the animals drinking free water. The basis for this proposal was that the total waterloss (transpirational and excretory) during the diuretic period was more than twice the total water gained by the spider through feeding on the prey. This suggested dependence of the diuretic response on the availability of drinking water was confirmed by comparing the volume of urine produced by hydrated spiders fed a single meal with and without water.

Two groups of hydrated animals of similar mean size (mean weights of groups,  $\pm$  S.E.M.,  $0.7065 \pm 0.1$  gm and  $0.7808 \pm 0.05$  gm) were starved with water for two days, then transferred to clean containers. One group was provided with a meal plus a continual supply of water throughout the experiment. The second group was provided with a meal of similar size but deprived of water throughout the experiment. All animals ingested the meal within 12 hours, and subsequent urine excretion was monitored for a further four days.

The mean daily rates of urine excretion of the two groups of fed animals, and that of a group of starved water deprived animals are shown in Figure IV.1. Animals provided with a meal plus water produced urine at an elevated rate ( $18-20 \mu\text{l day}^{-1}$ ) for two days, including the day of feeding. After the two day diuretic period urine excretion returned to a level equivalent to that observed in hydrated starved animals (Section I). In contrast, during the first two days of the experiment the fed, water deprived animals produced urine at a much reduced rate ( $6-8 \mu\text{l day}^{-1}$ ), and then the rate of excretion gradually declined to zero over the next three days. Significantly, however, the urine excretion of the fed, water deprived animals was greater than that of the starved, water deprived animals.

In Table IV.1 the total volumes of urine excreted by these hydrated animals are shown. Also shown are the total volumes of urine excreted by spiders which were deprived of water for varying lengths of



Table IV.1 The effect of water deprivation and dehydration on the total volume of urine excreted by spiders during and following a meal. All values  $\bar{X} \pm \text{S.E.M.}$

Duration of dehydration	Weight*	Total volume of urine excreted as either	
		$\mu\text{ls}$	OR a percentage of total water gained from the prey
Nil, hydrated fed with water, n = 8	100	$54.1 \pm 6.2$	$81.6 \pm 15$
Nil, hydrated but fed without water, n = 6	100	$18.6 \pm 7.5$	$16.3 \pm 6.3$
12 hours, n = 6	$97.5 \pm 0.5$	$13.2 \pm 3.2$	$13.4 \pm 4.0$
48 hours, n = 8	$93.6 \pm 1.3$	$12.0 \pm 4.2$	$15.3 \pm 5.5$
72 hours, n = 4	$91.2 \pm 1.8$	$12.4 \pm 3.1$	$9.5 \pm 2.2$
96 hours, n = 10	$89.2 \pm 2.3$	$4.0 \pm 0.8$	$3.4 \pm 1.6$

\* Weight expressed as a percentage of bodyweight when water was withheld.

time before feeding. These animals were taken from culture, starved with water for two days, then transferred to clean containers and deprived of water for periods of between 12 and 96 hours before they were fed a single meal without water. These animals were of a similar size as the hydrated animals and were fed a meal of similar size. The total volume of urine excreted by the animals is expressed as  $\mu$ ls and also as a percentage of the fluid volume gained by ingestion of the prey. This fluid volume was estimated by assuming that equal proportions of the total dry material and water in the prey would be ingested by the spiders during a meal (Section I). Although loss of water from the prey by evaporation during feeding occurs (Section IV.3.3) and so the assumption is not strictly true, the effect will be of similar magnitude for each group. Therefore comparisons made between groups will be valid.

Depriving animals of water under similar conditions in Section IV.3.4 resulted in a reduction of the water content of the animals. Thus effectively the animals here, which were deprived of water for varying periods prior to feeding, were dehydrated to different degrees. The relative extent of dehydration is indicated in Table IV.1 by their bodyweights which are expressed as a percentage of their bodyweight before the removal of water.

From Table IV.1 it can be seen that the largest reduction in the total volume of urine excreted resulted from depriving hydrated animals access to free drinking water during and after feeding. The volume of urine excreted by these animals decreased from 81% to 16% of the volume of fluid gained through ingestion of the prey. Dehydration prior to feeding resulted in further small reductions in the total volume of urine excreted, but these reductions were small compared to the initial effect of water deprivation during and after feeding.

#### IV.3.1 (b) The effect of water deprivation on the composition of the stercoral fluid

Summarised in Table IV.2 are measurements of the composition of the haemolymph and stercoral fluid of spiders maintained on four different dietary regimes:

- (i) Starved without water for six days.
- (ii) Fed without water for six days.

Table IV.2 The composition of the haemolymph and stercoral fluid of spiders maintained on different dietary regimes. All values  $\bar{X} \pm \text{S.E.M.}$  Values for hydrated animals from Section I.

		Osmotic pressure mOsm	Na mM l <sup>-1</sup>	K mM l <sup>-1</sup>	Cl mM l <sup>-1</sup>	Na/K
Starved without water for 6 days, n = 9*	Haemolymph	579.7 $\pm$ 15.5	297 $\pm$ 8.4	7.0 $\pm$ 0.1	-	-
	Stercoral fluid	-	-	-	-	-
Fed without water for 6 days, n = 10*	Haemolymph	615 $\pm$ 22.5 <sup>†††</sup>	287 $\pm$ 19.3	6.4 $\pm$ 0.15	-	-
	Stercoral fluid	620 $\pm$ 5.6	22.8 $\pm$ 5.8	190.6 $\pm$ 5.8	164 $\pm$ 20.2	0.16 $\pm$ 0.05
Starved with water for 5 days, n = 8	Haemolymph	436 $\pm$ 5.5	196 $\pm$ 3.9	5.08 $\pm$ 0.2	155.7 $\pm$ 4.8	-
	Stercoral fluid	427 $\pm$ 5.5	50.5 $\pm$ 11.5	64.3 $\pm$ 12.1	56.5 $\pm$ 12.9	1.04 $\pm$ 0.28
Fed with water for 5 days, n = 9	Haemolymph	515 $\pm$ 9.9	226 $\pm$ 5.3	4.5 $\pm$ 0.26	225 $\pm$ 2.7	-
	Stercoral fluid	504 $\pm$ 9.6	34.6 $\pm$ 8.1	125.4 $\pm$ 6.1	78.3 $\pm$ 8.0	0.28 $\pm$ 0.07

\* All values for animals deprived of water (both fed and starved) are significantly greater ( $0.05 > p$ ) than equivalent values for hydrated animals (both fed and starved), except the Na concentration of the stercoral fluid of animals deprived of water while feeding. (Unpaired Student t-test).

††† Indicates that the mean value for animals feeding without water are significantly different from mean values for animals starved without water. Unpaired Student t-test. Level of significance: †,  $0.05 > p > 0.01$ ; ††,  $0.01 > p > 0.001$ ; †††,  $p > 0.001$ .

Figure IV.2 The variation in the osmotic pressure of the stercoral fluid with changes in the osmotic pressure of the haemolymph.

- Hydrated starved animals.
- Hydrated fed animals.
- Dehydrated fed animals.

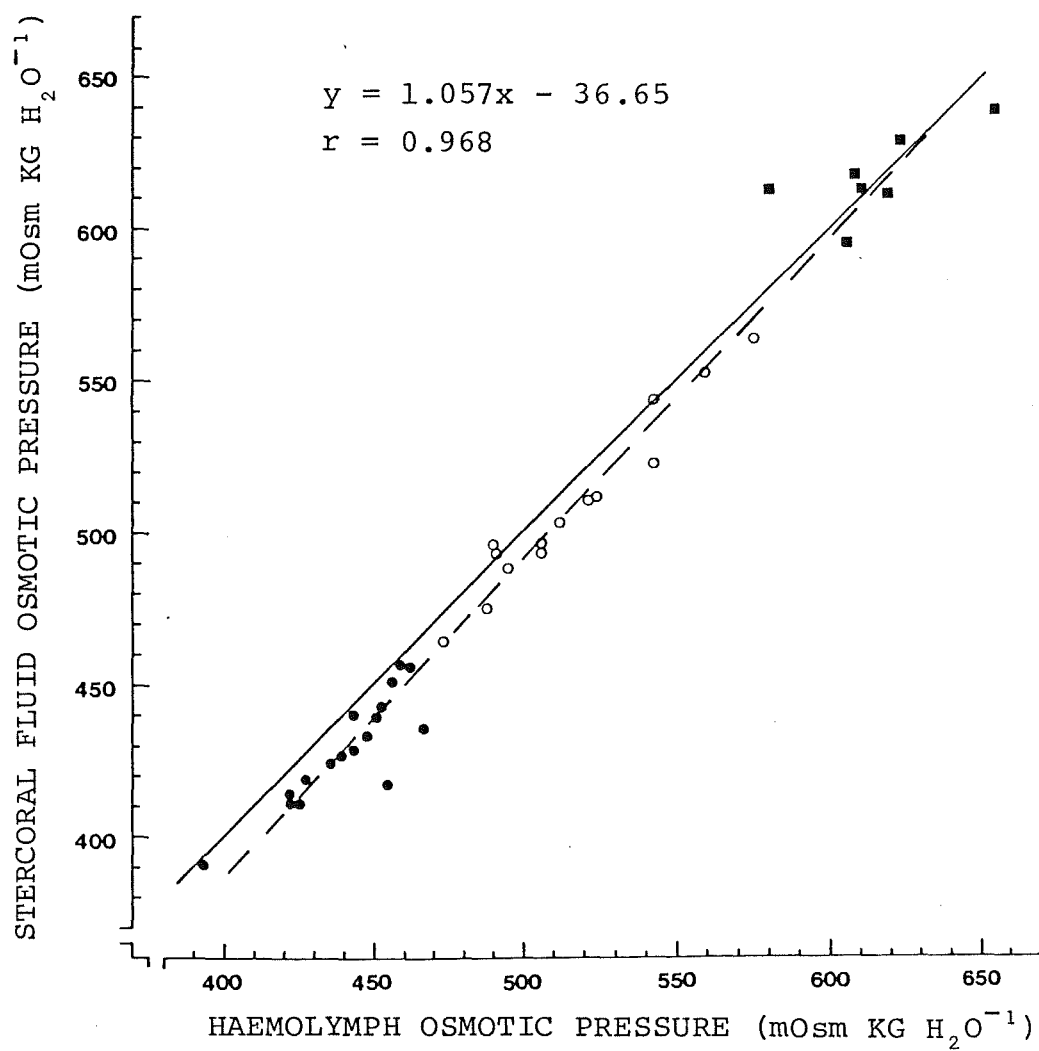
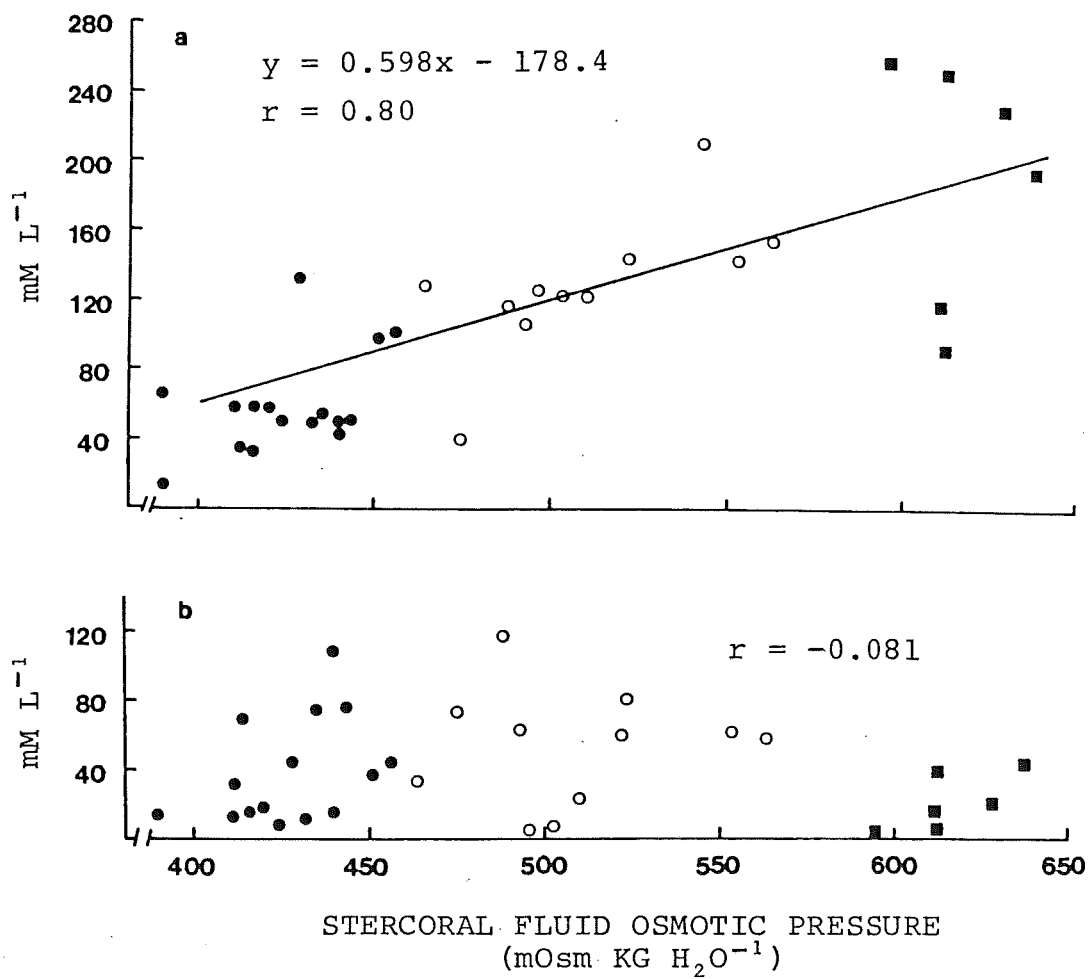


Figure IV.3 Variation in the Na and K concentration of the stercoral fluid with increasing osmotic pressure of the fluid. (a) K, (b) Na.

- Hydrated starved animals.
- Hydrated fed animals.
- Dehydrated fed animals.



- (iii) Starved with water for five days (Section I).
- (iv) Fed with water for five days (Section I).

Prior to the 5-6 day period of feeding or starvation animals were starved with water for three weeks. Attempts to collect stercoral fluid from dehydrated starved animals were unsuccessful.

The most significant point arising from this table is that the stercoral fluid of the animals deprived of water during feeding was essentially iso-osmotic to the haemolymph. It appears that *P. antipodiana* is incapable of producing a final urine either markedly hyper-osmotic or, for that matter, even markedly hypo-osmotic to the haemolymph. In Figure IV.2 the osmotic pressure of the stercoral fluid collected from spiders maintained on the three different regimes is plotted against the osmotic pressure of the haemolymph. Over the entire range of haemolymph osmotic pressures encountered in *P. antipodiana* the stercoral fluid was essentially iso-osmotic to the haemolymph. The small degree of hypo-osmoticity apparent in the hydrated starved animals, while being statistically significant (Section I, paired Student *t* test), is of little physiological significance to the animals, the degree of hypo-osmoticity being insufficient to produce a fluid of significant dilution when compared to the haemolymph.

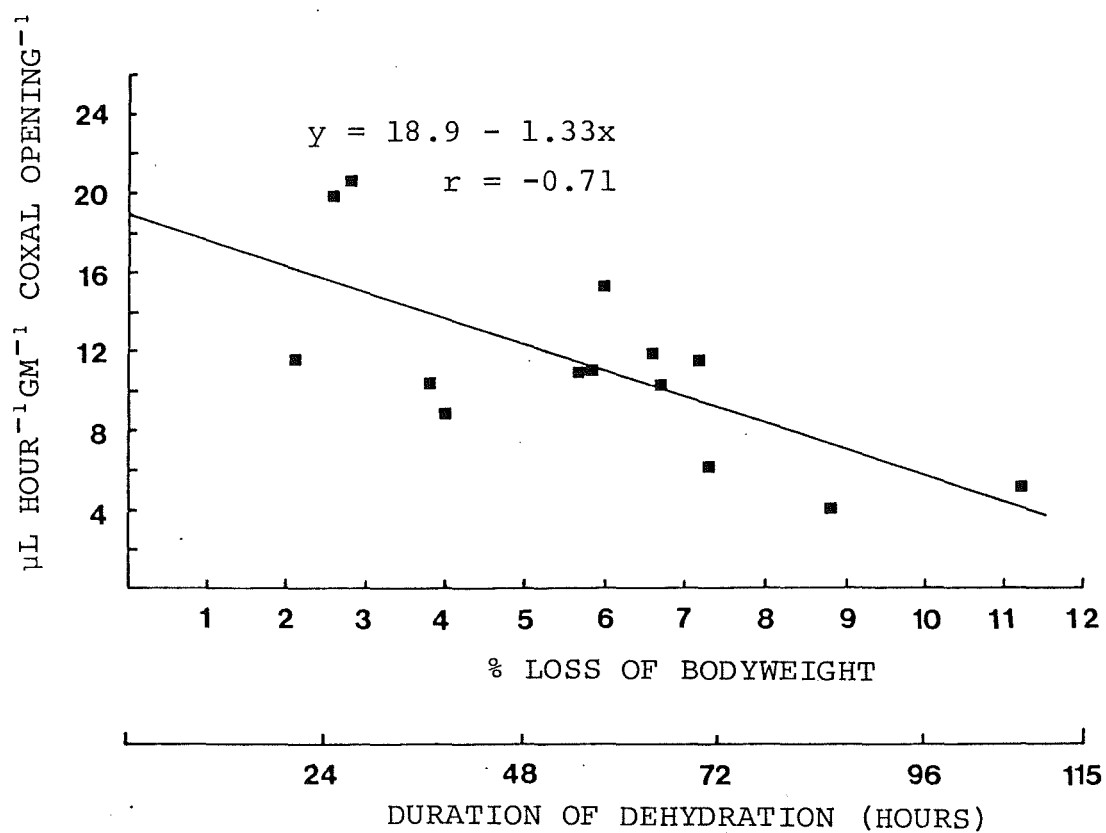
Also of particular interest is that the Na concentration of the stercoral fluid of water deprived, fed animals ( $22.8 \text{ mM l}^{-1}$ ) was less than that of either hydrated fed ( $35 \text{ mM l}^{-1}$ ) or hydrated starved animals ( $50.5 \text{ mM l}^{-1}$ ). It appears that there is little regulation of the stercoral fluid Na concentration (Figure IV.3). Conversely, the K concentration of the stercoral fluid varies quite significantly between the groups, and increased with the osmotic pressure of the stercoral fluid (Figure IV.3).

#### IV.3.2 The Functioning of the Coxal Glands of Dehydrated Spiders

To investigate changes in the rate of coxal excretion and the composition of the coxal fluid with dehydration, a number of spiders were deprived of water and dehydrated for varying periods of time, and then coxal fluid collected as described in Section II with the modifications noted below. It was immediately obvious that dehydrated spiders would



Figure IV.4    Change in the rate of coxal excretion with increasing dehydration.    Each point represents a value for an individual spider.    Solid line represents regression curve fitted for points by least squares method.



not feed as readily as their hydrated counterparts, particularly if they had lost in excess of 10% of their bodyweight through dehydration. Increased success in inducing restrained spiders to feed was achieved if inulin injections and haemolymph samples were not carried out before feeding.

#### IV.3.2 (a) The rate of coxal excretion

As with hydrated spiders the onset of coxal excretion of dehydrated animals coincided with the start of feeding and ceased with the completion of the meal. Dehydration did, however, result in a reduction in the mean rate of coxal excretion (expressed as  $\mu\text{l hr}^{-1} \text{ gm}^{-1}$  coxal opening<sup>-1</sup>), increasing dehydration resulting in a progressive decline in the rate of excretion (Figure IV.4). One would perhaps expect some reduction in the rate of excretion due to the difficulty in inducing the animals to feed. However, the rate of excretion was only measured for those spiders which began feeding immediately and continued to feed throughout the experiment, thus the observed decline in the rate is likely to be related to the reduced hydration state of the animals, rather than an artefact arising from the reluctance of the dehydrated animals to feed.

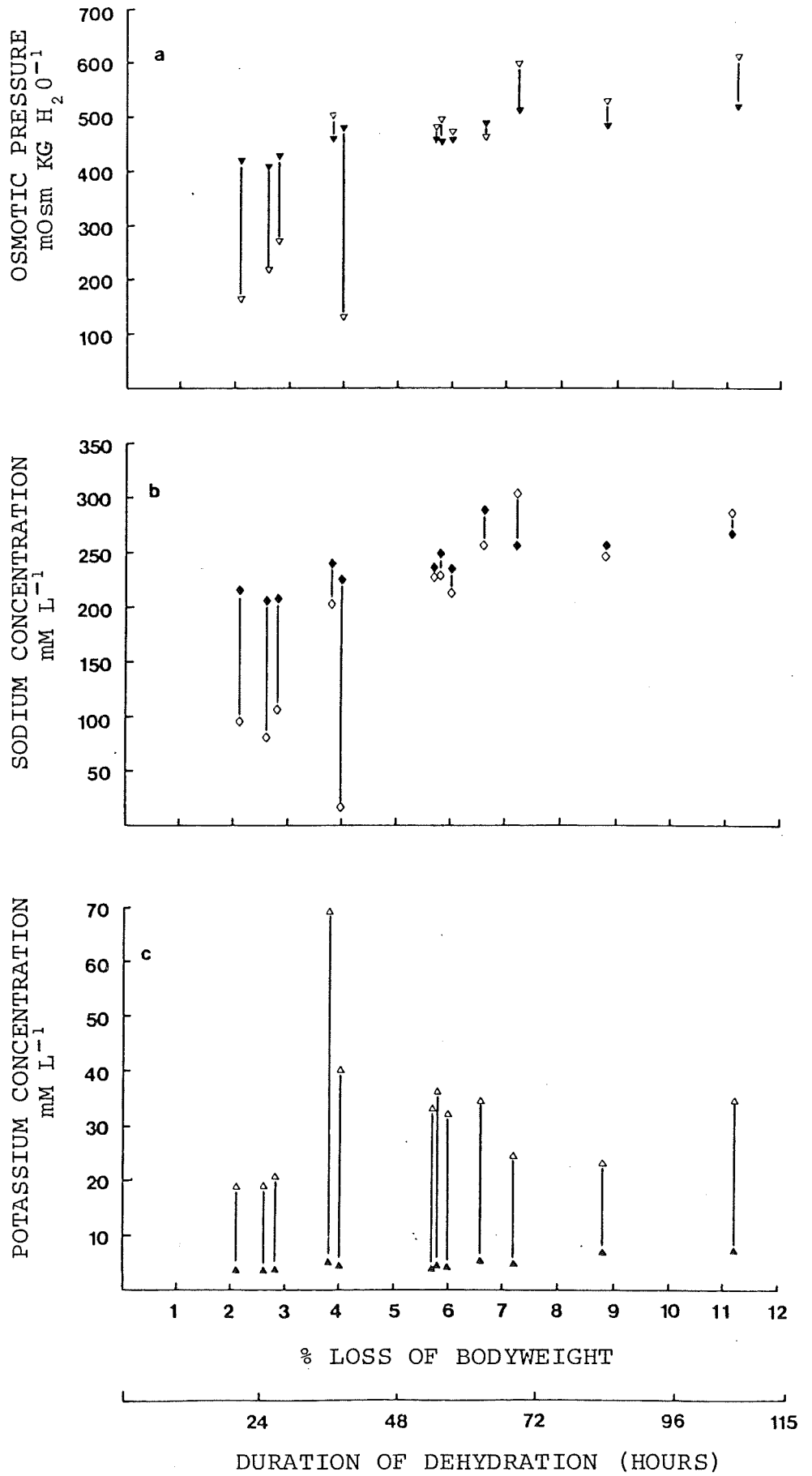
Unfortunately, animals which had lost in excess of 12% of their bodyweight through dehydration would not feed when restrained, thus it could not be established whether spiders would feed in the absence of coxal excretion. Unrestrained spiders which had lost similar proportions of their bodyweight would feed, although at times reluctantly.

#### IV.3.2 (b) Composition of coxal fluid excreted by dehydrated animals

Shown in Figure IV.5 is the osmotic pressure, Na and K concentrations of the coxal fluid and haemolymph of spiders dehydrated for varying periods of time. Haemolymph measurements are from samples collected after the completion of the meal and collection of coxal fluid.

As would be predicted from results presented in Section III, dehydration resulted in increases in the osmotic pressure, Na and K concentrations of the haemolymph.

Figure IV.5 Changes in the composition of the haemolymph and coxal fluid composition with increasing dehydration. (a) osmotic pressure. (b) Na concentration. (c) K concentration. Open symbols = coxal fluid, closed symbols = haemolymph. Vertical lines link haemolymph and coxal fluid values from same animal.



With regards to the coxal fluid, in animals dehydrated for brief periods (24 - 36 hours) the osmotic pressure of the coxal fluid remained markedly hypo-osmotic to the haemolymph. However, animals which were dehydrated for further periods excreted coxal fluid which was iso-osmotic or even somewhat hyper-osmotic to the haemolymph, although as with the stercoral fluid the coxal fluid was never markedly hyper-osmotic to the haemolymph. Changes in the Na concentration of the coxal fluid closely paralleled changes in the osmotic pressure, but there were no predictable changes in the K concentration of the coxal fluid with dehydration, and although it was quite variable the mean K concentration of the coxal fluid of dehydrated spiders ( $32.1 \pm 4$ ;  $\bar{X} \pm \text{S.E.M.}$ ,  $n = 12$ ) was not significantly different ( $0.5 > p > 0.1$ ) to that observed for hydrated spiders ( $35.6 \pm 2.9$ ;  $\bar{X} \pm \text{S.E.M.}$ ,  $n = 12$ ) (Section II).

#### IV.3.3 The Ion and Water Balance of Dehydrated Fed Spiders

Two groups of spiders of similar mean weights (control animals,  $0.8834 \pm 0.037$  gm; experimental animals,  $0.8848 \pm 0.033$  gm;  $\bar{X} \pm \text{S.E.M.}$ ,  $n = 14$  for each group) were labelled with  $^{22}\text{Na}$  and then after 48 hours recovery and equilibration transferred to containers without water. After three days dehydration the experimental animals were provided with a meal of known composition (Section I) for 24 hours. They were then dehydrated for a further three days before being provided with free water for rehydration. Control animals were dehydrated and rehydrated in exactly the same manner as the experimental animals but were not fed at any stage. At the completion of the dehydration period, eight animals from each group were killed and their total Na and K content determined. The weight of the animals, the production of urine by the anal system, and the excretion of Na and K by the anal system and the coxal glands were all measured daily.

##### IV.3.3 (a) Water balance of dehydrated spiders

The rates of anal and coxal fluid excretion are summarised in Figure IV.6. As was seen in Section III, with dehydration of starved spiders the rate of anal urine excretion progressively declines to zero. The provision of food to the dehydrated spiders resulted in a small diuretic response during the feeding day plus one subsequent day. The total urine output in the two day period was  $13.2 \mu\text{l}$ , the highest mean daily rate of excretion occurring on the day following feeding

Figure IV.6 The rate of fluid production by the coxal glands and the anal system during periods of dehydration and rehydration and the effect of a meal during the dehydration period. Experimental animals dehydrated for three days, fed for 24 hours then dehydrated for a further three days, before rehydration. Control animals starved throughout the experiment.

△---△ Urine excretion of controls.

▲——▲ Urine excretion of experimental animals.

□---□ Coxal excretion of controls.

■——■ Coxal excretion of experimental animals.

During the dehydration period  $n = 14$ , during rehydration  $n = 6$ .  
All values  $\bar{X} \pm 1 \text{ S.E.M.}$

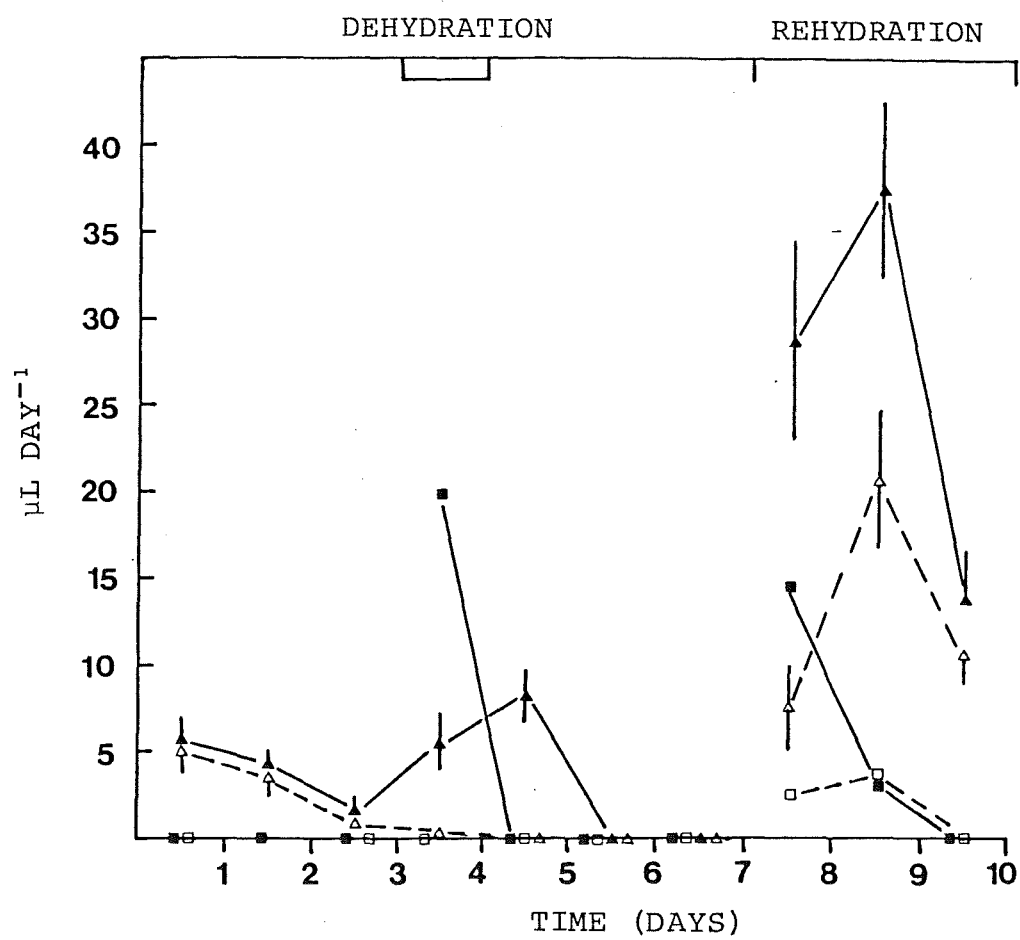




Table IV.3 The water balance of *P. antipodiana* provided with a single meal without free water after three days of dehydration. All gains and losses of water were measured during the two day period of feeding and excretion.

	Total ingested	Transpirational* loss	Anal ** excretion	Coxal excretion	Total loss
$\mu\text{ls}$	97	44	13.2	20	77
Percent of water ingested	100	45	14.0	21	80

\* Transpirational loss determined at a rate of  $22 \text{ mg day}^{-1}$  for the two day diuretic period.

\*\* Anal excretion measured over a two day period of diuresis.

(8.5  $\mu\text{l day}^{-1}$ ). This contrasts with the diuretic response seen in hydrated animals (Section I) which lasted at least three days during which the rate of urine excretion was maintained at between 20 and 25  $\mu\text{l day}^{-1}$ .

Coxal excretion was determined by locating regions of  $^{22}\text{Na}$  activity on the filter paper lining the experimental chambers or in the prey debris. There was no evidence of coxal excretion by the starved animals throughout the dehydration period and the fed animals only excreted coxal fluid into the prey. This was apparent from the high  $^{22}\text{Na}$  content of the prey debris and the fact that the Na/K ratio of the prey debris,  $0.77 \pm 0.06$  ( $\bar{X} \pm \text{S.E.M.}$ ,  $n = 14$ ), was higher than that of the whole food provided,  $0.46 \pm 0.02$  ( $\bar{X} \pm \text{S.E.M.}$ ,  $n = 20$ ).

There was no means to directly measure the volume of coxal fluid excreted into the prey by the dehydrated animals, however, an estimate of the amount of coxal fluid remaining in the prey on completion of the meal can be made from the calculated K loss from the spider into the prey and the K concentration of the coxal fluid which does not increase with dehydration (Section IV.3.2). The volume of coxal fluid calculated in this manner was 20  $\mu\text{l}$ , a smaller volume than was lost by hydrated spiders (37  $\mu\text{l}$ ) when feeding on a meal of similar size (Section I).

The water balance sheet drawn up for this single meal and the excretion associated with it is presented in Table IV.3. Dehydrated feeding animals have only one source of water, the prey. Losses occur through excretion, both coxal and anal, and transpiration. The amount of water gained through feeding was calculated from the following relationship -

$$\text{Water ingested} = W_t + T + E - D$$

where

$W_t$ = total weight gained through feeding	80 mg
$T$ = transpirational loss during feeding	22 mg
$E$ = excretory waterloss during feeding (coxal and anal)	26 mg
$D$ = weight gain due to the ingestion of dry material	<u>31 mg</u>
Water ingested	<u>97 mg</u>

No allowance was made for the small amount of dry material excreted by the animals.

Calculation of the quantity of water ingested by feeding animals here differs from the method employed in Sections I and IV.3.1(a) above, where ingested water was calculated assuming that equal proportions of total dry material and water in the prey would be ingested by the spiders during a meal. It was noted in Section I and above that this assumption is not necessarily precise as evaporation of water from the prey will occur during feeding. The significance of this evaporative waterloss can be seen by comparing estimates of the quantity of water gained from the prey by dehydrated spiders calculated by the two different methods. Based on the assumption that similar proportions of dry material and water were ingested by the dehydrated spiders it was calculated that 111 mg of fluid was gained from the prey, whereas employing the relationship which considers gains and losses of weight it was estimated that 97 mg of fluid were gained from the prey.

Transpirational loss during and after feeding was estimated from the rates of waterloss of the control animals during the same interval. Coxal and anal waterloss was determined as described above.

The significant point arising from the water balance sheet is that the dehydrated spiders achieved a net gain of water from the prey, due essentially to the reductions in excretory waterlosses. If the volumes of coxal fluid and urine produced by the dehydrated spiders are compared to those produced by hydrated spiders of similar size and ingesting a meal of similar size (Section I), it can be seen that coxal excretion by the dehydrated animals was almost halved (20  $\mu$ l compared to 37  $\mu$ l), and urine excretion reduced from a total of 93  $\mu$ l (101% of the ingested water) to 13.2  $\mu$ l (14% of the ingested water).

Interestingly, during rehydration there was evidence of coxal excretion by both groups of animals. This is of some significance as it is the second example of coxal excretion at times other than feeding and confirms that although the excretion of coxal fluid is involved in the feeding process, the coxal glands also play an excretory role. It was also notable that during the diuretic period associated with the rehydration (Figure IV.6) animals which were fed during the dehydration period excreted both urine and coxal fluid at greater rates than the starved controls.

Figure IV.7 The rate of Na and K excretion by the anal system and the coxal glands during periods of dehydration and rehydration and the effect of a meal during the dehydration period. Experimental animals dehydrated for three days, fed for 24 hours then dehydrated for a further three days before rehydration. Control animals starved throughout the experiment. (a) Na excretion. (b) K excretion. (c) Na/K ratio of coxal fluid. (d) Na/K ratio of the excreta.

△---△ Anal excretion by controls (excreta).

▲——▲ Anal excretion by experimental animals (excreta).

□---□ Coxal excretion by controls.

■——■ Coxal excretion by experimental animals.

During dehydration  $n = 14$ , during rehydration  $n = 6$ . All values  $\bar{X} \pm 1 \text{ S.E.M.}$

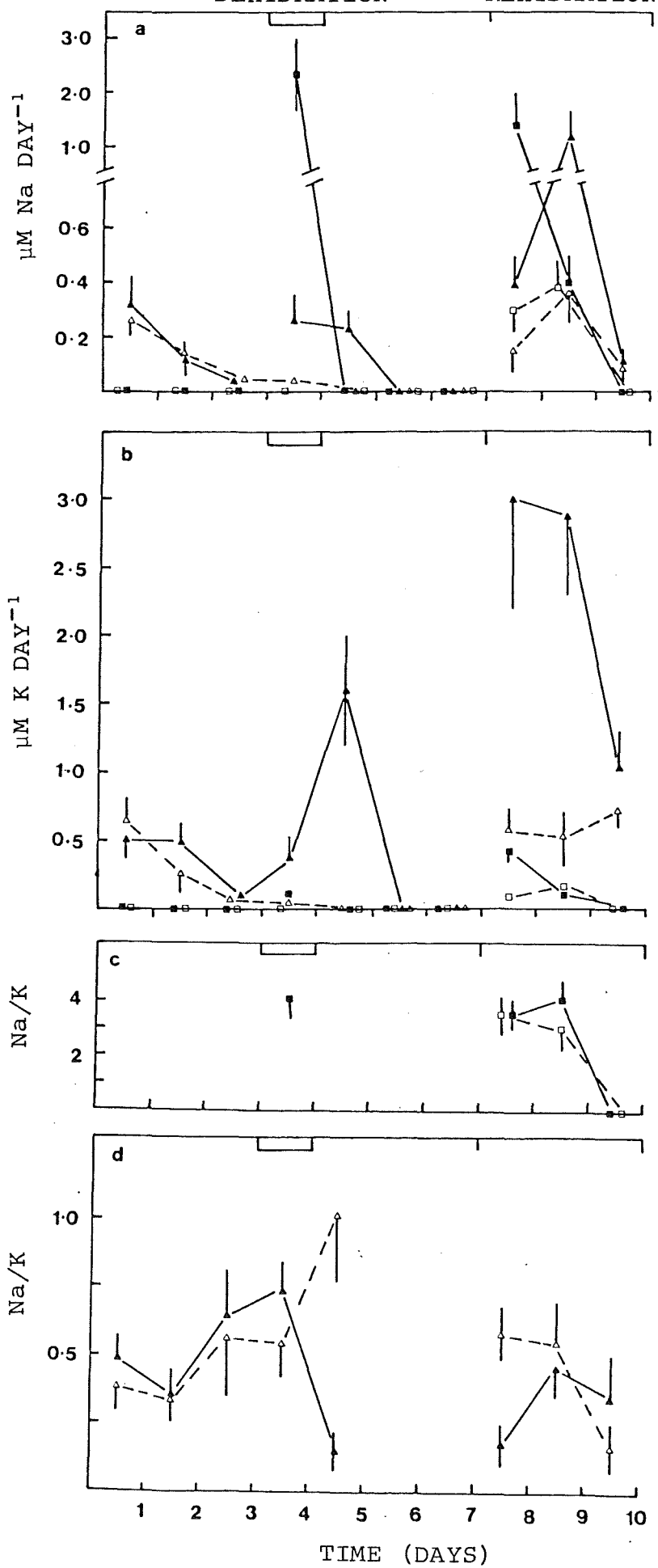


Table IV.4 The ion balance of *P. antipodiana* provided with a single meal without free water after three days of dehydration.

	Total ingested	Anal excretion*	Coxal excretion*	Total excretion*	Cation content of starved animals	Cation content of fed animals
$\mu\text{M}$	7.3	0.64	2.24	2.9	62.5	67.8
Sodium						
Percent of ingested	100	9.0	31.0	40	-	-
$\mu\text{M}$	17.8	1.82	0.53	2.4	49.3	67.5
Potassium						
Percent of ingested	100	10.2	3.0	13.2	-	-

\* Excretion during dehydration period only.

### IV.3.3 (b) Ion balance of dehydrated fed spiders

The rates of Na and K excretion by the anal system and the coxal glands of spiders fed a single meal during dehydration are shown in Figure IV.7. There was a decrease in both Na and K excretion via the anus with dehydration, however, during the brief period of diuresis observed during and following feeding increases in both Na and K excretion via the anus were evident. The maximum daily anal Na excretion rate ( $0.26 \mu\text{M day}^{-1}$ ) occurred on the day of feeding and was considerably less than the maximum anal K excretion ( $1.6 \mu\text{M day}^{-1}$ ) which occurred the day after feeding. It is probable that initial anal Na and K excretion would be influenced by the contents of the stercoral pocket and thus not necessarily representative of feeding. Changes in the Na/K ratio of the excreta voided via the anus of dehydrated animals support this proposal (Figure IV.9). The excreta voided on the day of feeding had a Na/K ratio of 0.74, a value more representative of starved animals, whereas following feeding the Na/K ratio decreased to 0.15, a value representative of feeding animals.

Coxal excretion during feeding was of some importance with respect to the elimination of Na. A total of  $2.3 \mu\text{M Na}$  were lost from the spider into the prey debris as a result of coxal excretion. This compares with the excretion of only  $0.64 \mu\text{M Na}$  via the anus during and following the meal. Conversely, the coxal glands were relatively unimportant in the elimination of K, excreting only  $0.53 \mu\text{M K}$  into the prey debris compared with the excretion of  $1.82 \mu\text{M K}$  by the anal system during and following feeding.

The ion balance for the dehydrated fed animals is summarised in Table IV.4. The animals ingested  $7.3 \mu\text{M Na}$  and  $17.8 \mu\text{M K}$  during feeding. However, only 40% ( $2.9 \mu\text{M Na}$ ) of the ingested Na and 13.2% ( $2.4 \mu\text{M K}$ ) of the ingested K were excreted by the coxal glands and the anal system during and following the meal. The remaining Na and K accumulated in the spiders. Therefore, although *P. antipodiana* achieved a net gain of water from the prey, it was not osmotically free water, and at the completion of the two day period of feeding and excretion the net result was that the spider had ingested a K rich, hyper-osmotic fluid (i.e.,  $15.4 \mu\text{M K}$  and  $4.4 \mu\text{M Na}$  in  $20 \mu\text{l}$  of water).

Table IV.5 The ion balance of spiders fed a single meal during dehydration, and then subsequently rehydrated. The proportion of ingested ions excreted during and following the meal in the dehydration period, and the proportion of ingested ions excreted during the rehydration period.

	Total ingested	Total excretion during dehydration (coxal and anal)	Excretion during rehydration resulting from meal			Total excretion resulting from meal
			Coxal	Anal	Total	
$\mu\text{M}$	7.3	2.9	1.1	1.12	2.22	5.12
Sodium						
% of ingested	100	40.0	15.0	15.3	30.3	70.3
$\mu\text{M}$	17.8	2.4	0.35	5.0	5.4	7.8
Potassium						
% of ingested	100	13.2	2.0	28.0	31.0	43.2



#### IV.3.3 (c) The effect of rehydration on the ion balance of spiders fed during dehydration

It was noted above that during the rehydration period the coxal glands and the anal system of spiders fed during dehydration produced fluid at greater rates than the two systems in the control spiders which were starved during dehydration. Associated with this increased fluid output was an increase in the rate of Na and K excretion (Figure IV.7). As noted for the excretion observed during dehydration, the coxal glands were an important route for the elimination of Na but not K, and the anal system excreted larger amounts of K relative to Na.

This increased rate of Na and K excretion observed in the dehydrated fed spiders was not unexpected, these animals having accumulated large amounts of ions from the meal ingested during dehydration, and one would conclude that these elevated rates of excretion involve the elimination of these excess ions. This conclusion is supported by the ion balance sheet presented in Table IV.5. This balance sheet includes excretion during and after the meal in the dehydration period and excretion in the rehydration period which results from this meal (i.e., the difference between the amounts of Na and K excreted by rehydrating fed animals and rehydrating starved animals). From the table it can be seen that of the Na and K ingested in the meal taken during the dehydration period, after the diuresis during rehydration a total of 70% ( $5.1 \mu\text{M Na}$ ) of the Na and 43% ( $7.75 \mu\text{M K}$ ) of the K were excreted. Similar proportions of ingested Na and K were excreted by hydrated fed animals (Section I). Furthermore, as with the hydrated animals, most of the excreted Na (65%) was eliminated by the coxal glands whereas most of the excreted K (89%) was eliminated via the anus.

#### IV.3.4 Continuous Feeding Without Water

Two groups of spiders of similar mean weight (control group,  $0.7357 \pm 0.053$  gm; experimental group,  $0.7047 \pm 0.0429$  gm;  $\bar{X} \pm \text{S.E.M.}$ ,  $n = 10$  for each group) were transferred to clean containers without water after three weeks starvation with water. Experimental animals were provided with sufficient live prey to allow them to feed at will, and the control animals starved. On each day the number of animals which fed, the weights of each of the animals, and the rates of anal urine excretion and Na and K production in each animal were measured. Coxal

Figure IV.8 Effect of water deprivation on the frequency of feeding by *P. antipodiana*. Both groups of spiders were provided with sufficient prey to allow them to feed at will.

- Spiders feeding without access to drinking water, n = 10.
- Spiders feeding with access to drinking water, n = 9.

Figure IV.9 Changes in the bodyweight of spiders provided with food with and without access to drinking water.

- Spiders feeding at will and provided with drinking water, n = 9\*.
- ▼——▼ Feeding spiders deprived of drinking water, n = 10.
- Starved spiders provided with drinking water, n = 8\*.
- ▽---▽ Starved spiders deprived of drinking water, n = 10.

\* Values from Section I.

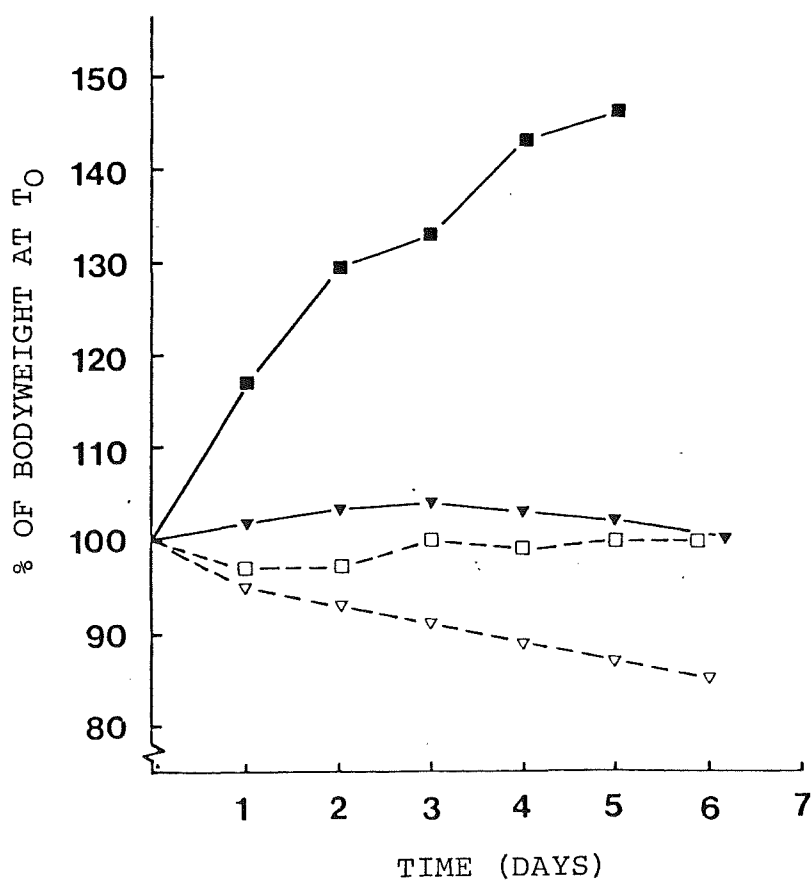
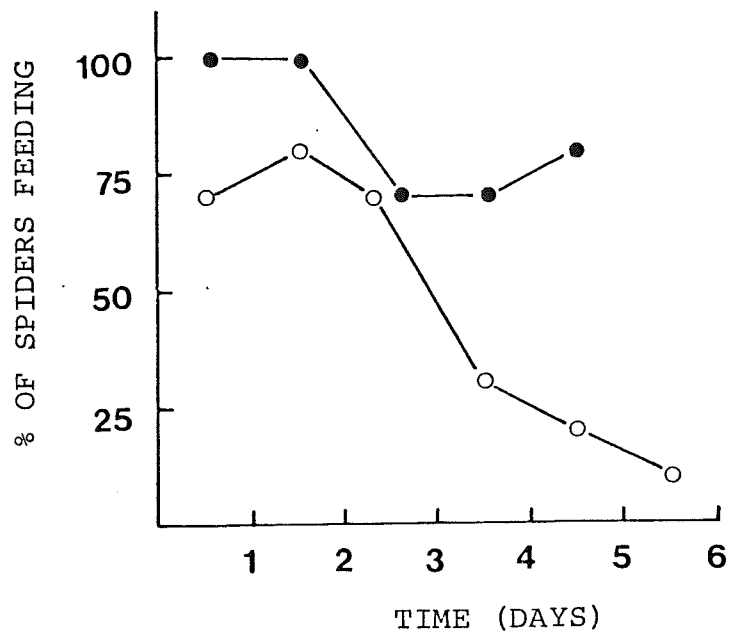


Fig. IV.10 Effect of water deprivation during feeding on the rate of urine excretion of *P. antipodiana*. Feeding spiders provided with sufficient food to allow them to feed at will. All values  $\bar{X} \pm 1 \text{ S.E.M.}$

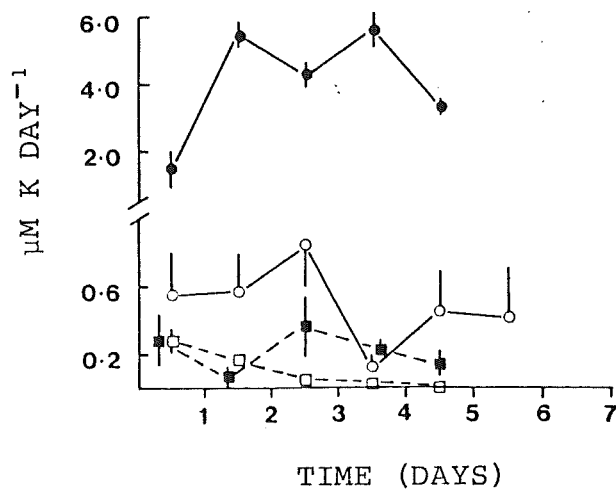
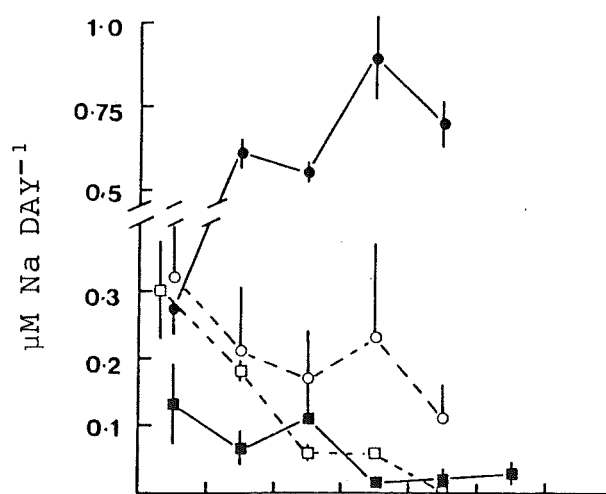
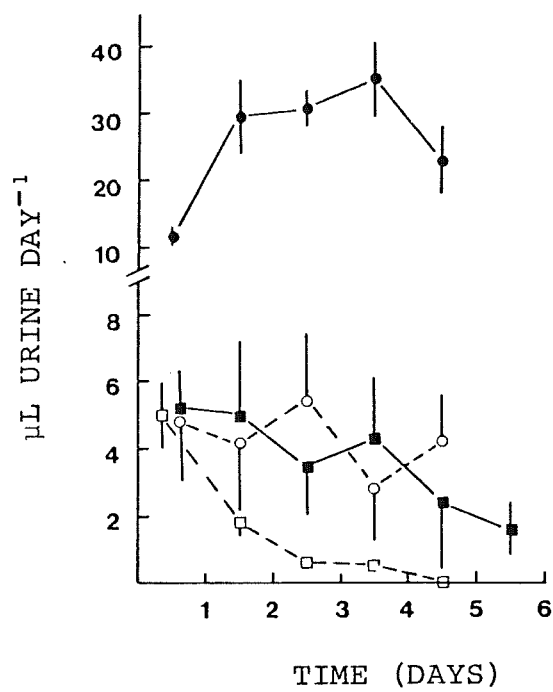
- Spiders feeding at will and provided with drinking water,  $n = 9^*$ .
- Spiders feeding at will but deprived of drinking water,  $n = 10$ .
- Starved spiders provided with drinking water,  $n = 8^*$ .
- Starved spiders deprived of drinking water,  $n = 10$ .

\* Values from Section I.

Fig. IV.11 Effect of water deprivation during feeding on the rate of anal Na and K excretion by *P. antipodiana*. Feeding spiders provided with sufficient food to allow them to feed at will. (a) Na excretion. (b) K excretion. All values  $\bar{X} \pm 1 \text{ S.E.M.}$

- Spiders feeding at will and provided with drinking water,  $n = 9^*$ .
- Spiders feeding at will but deprived of drinking water,  $n = 10$ .
- Starved spiders provided with drinking water,  $n = 8^*$ .
- Starved spiders deprived of drinking water,  $n = 10$ .

\* Values from Section I.



excretion was not measured. After six days of feeding without water, haemolymph and stercoral fluid samples were collected, the animals killed and their water content determined.

Initially the animals fed regularly, however, after three days the number of animals feeding each day declined markedly and continued to decline as the experiment progressed (Figure IV.8). Consequently, although the mean weight of the feeding group was maintained in the initial three days of the experiment thereafter it declined, although at a slower rate than that of the starved animals (Figure IV.9).

The rate of urine excretion of the animals fed without water was much reduced when compared to that of animals fed with water (Section I and Figure IV.10). Spiders feeding at will with access to drinking water consistently produced urine at rates as high as  $32 \mu\text{l day}^{-1}$ , whereas the water deprived animals produced urine at a maximum rate of  $5.4 \mu\text{l day}^{-1}$  during the first day and thereafter the rate of urine excretion gradually declined. Not unexpectedly the rate of anal Na and K excretion was also much reduced in the animals feeding without water (Figure IV.11).

Both starved and fed animals which were deprived of water showed reductions in their relative water contents (Table IV.6). However, the decrease in the relative water content of the starved animals was due to a large reduction in their total water content (-22.5%), whereas in the feeding animals there was only a 9% reduction in their water content but also a 25% increase in their dry material.

Table IV.6 Changes in the relative water content, dry weight and water content of spiders deprived of water and either allowed to feed at will or starved. All values  $\bar{X} \pm \text{S.E.M.}$  Values for hydrated starved controls from Section I.

Treatment	Relative water content	Percentage change from time zero	
		Dry weight	Wet weight
Starved hydrated (n = 8)	$74.8 \pm 0.74$	-	-
Starved dehydrated (n = 10)	$68.3 \pm 1.7$	$-1.0 \pm 0.2$	$-22.5 \pm 1.8$
Fed dehydrated (n = 10)	$67.4 \pm 2.6$	$25.1 \pm 3.9$	$-9.87 \pm 1.0$

#### IV.4 DISCUSSION

As spiders are, in effect, fluid feeders, initial considerations might have led one to suspect that spiders feeding *ad libitum* would require no additional water source. However, for *P. antipodiana* this has been shown not to be the case.

Both the anal system and the coxal glands do in fact reduce their fluid output during and following a meal when *P. antipodiana* is fed during dehydration. The reductions are such that dehydrated spiders do actually achieve a net gain of water from their prey. However, neither the coxal glands nor the anal system can produce a fluid of sufficient concentration to eliminate, in the small volumes of fluid excreted, the ions ingested with the meal. Thus the net effect of feeding during dehydration is the ingestion of a fluid markedly hyper-osmotic to the haemolymph, and dehydrated animals which feed are forced into an ionic imbalance. The excess ions which a spider ingests during a meal can only be eliminated if free water is available during and following feeding (Section I). If feeding occurs during dehydration the ingested ions are eliminated when free water is later made available for rehydration, as in the experiments reported here. In this latter case both the coxal glands and the anal system participate in the excretion of ions. This example of coxal excretion without associated feeding confirms that the coxal glands should be considered as true excretory organs.

Probably as a result of the inability of the coxal glands and the anal system of dehydrated animals to excrete the ions ingested with feeding, water deprived animals cease to feed regularly. Spiders feeding *ad libitum* on live prey without water fed regularly for a period of three days, but thereafter the frequency of feeding declined markedly. This is in contrast to the hydrated spiders which fed regularly for the duration of the experiment.

For a terrestrial animal to survive on the water available in its food alone, it must produce a urine which is not only osmotically more concentrated than the food but is of sufficiently high concentration to offset any transpirational losses that occur during feeding and in the period before the next meal. Even though the rates of transpirational waterloss of *P. antipodiana* are quite low and comparable with xeric arthropods (Section III), nevertheless under the conditions of the experiments reported above such losses were a major component of the total water turnover. Furthermore, as



*P. antipodiana* is an opportunistic intermittently feeding predator, even under the more humid conditions of its natural environment transpirational losses between meals would still comprise a major component of the total water turnover. Thus although some spiders were observed here to produce excretory fluid which was marginally hyper-osmotic to the haemolymph (and hence even more hyper-osmotic to the body fluids of the cockroach prey) the degree of concentration is quite inadequate to deal with the transpirational losses of water.

Interestingly, the largest reduction in the total volume of urine produced by the dehydrated fed spiders arose from the initial deprivation of water. Hydrated spiders provided with access to free drinking water excrete copious quantities of urine following the meal (Section I and above). However, if the hydrated animals are deprived of water during and after feeding the total volume of urine produced is markedly reduced. Periods of dehydration prior to feeding result in little further reduction in the total volume of urine produced.

The rate of coxal excretion varies inversely with the hydration state of the animals. Reductions in the rate of excretion of filtration "kidneys" such as the coxal glands, may occur either by a reduction in the rate of filtration or by an increase in the post-filtration reabsorption of water from the urine. There is evidence that both mechanisms operate in arthropod filtration "kidneys". Bryan and Ward (1962) reported a clearance ratio (urine: haemolymph inulin ratio) as high as 31 for the freshwater crayfish, *Austropotamobius pallipes*, when it was maintained in 50% sea water, implying a considerable water reabsorption, and Greenaway (1981) estimated that the clearance ratio for the semi-terrestrial crab, *Holthuisana transversa* was as high as 15.7. Reductions in the rate of filtration, which produce reductions in the rate of final urine production, have been demonstrated in *Pacifastacus leniusculus* (Pritchard and Kerley, 1970), the terrestrial crab, *Gecarcinus lateralis* (Harris, 1977) and the terrestrial crabs, *Gecarcoidea lalandii*, *Cardisoma carnifex* and *Birgus latro* during periods of reduced availability of water (Kormanik and Harris, 1981; Harris and Kormanik, 1981). Without inulin clearance measurements for the coxal glands of the dehydrated spiders it is not possible, however, to determine which, or if both, mechanisms are responsible for the observed reductions in the rate of coxal excretion of dehydrated feeding *P. antipodiana*.

The inability of *P. antipodiana* to produce a hyper-osmotic fluid may

be a factor which limits its distribution in the terrestrial environment. Most terrestrial insects investigated to date are capable of producing a hyper-osmotic urine (Wigglesworth, 1931; Ramsay, 1952, 1955; Phillips, 1964b) and this ability to produce a concentrated urine is considered to be one of the features which accounts for their wide distribution and success (Phillips, 1970). However, there are reported examples of terrestrial arthropods which cannot produce a hyper-osmotic urine, e.g. the insect *Dysdercus fasciatus* (Berridge, 1965a) and the terrestrial crustaceans (Edney, 1968; Bliss, 1968; Harris and Kormanik, 1981).

In the terrestrial crustaceans osmotic regulation and water balance are generally achieved by behavioural mechanisms which serve to maintain the animals in a favourable environment and with access to free water (Edney, 1961, 1977; Bliss, 1968; Warburg, 1968), often by burrowing (Harris and Kormanik, 1981). The habitat of *P. antipodiana* would suggest that similar behavioural mechanisms are important in its water balance and osmoregulation. *P. antipodiana* is limited to damp environments and is often found in silk funnel like tunnels which extend up to 40 cm down among shingle and stones (Todd, 1945; Section III). Unlike the terrestrial crustaceans, transpirational waterloss from *P. antipodiana* is low and comparable to that of arachnids and insects found in xeric environments (Section III). Thus cuticle permeability in itself is not necessarily a major factor limiting the distribution of *P. antipodiana*.

In relatively hygric environments, such as that inhabited by *P. antipodiana*, an excess rather than a shortage of water might be expected. Thus the ability to produce a hypo-osmotic, rather than a hyper-osmotic, urine might be seen to be of more significance. The anal system appears rather poorly adapted for this role, as even in starved hydrated spiders the stercoral fluid is not markedly hypo-osmotic to the haemolymph. The coxal glands of hydrated spiders do produce a fluid which is hypo-osmotic to the haemolymph, and in this sense they are apparently suited to the elimination of excess water. However, the glands only excrete at a high rate in association with feeding. Hydrated starved spiders were not observed to produce coxal fluid and although rehydrating starved animals were observed to produce coxal fluid, the volume produced was small (3-5  $\mu\text{l day}^{-1}$  for the first two days of rehydration).

In the absence of an efficient excretory mechanism for the elimination

of excess water it is likely that tight control of drinking combined with evaporative waterlosses across the cuticle and respiratory surfaces is sufficient to prevent the accumulation of excess water by the spiders. It is also possible that the animals could shed excess water by increased evaporative losses brought about by behavioural mechanisms. In this respect it is significant that the permeability of winter spiders is some four times greater than that of summer spiders (Section III).

In both hydrated and dehydrated spiders salt excretion is divided between two routes; most of the Na excreted during and following a meal is eliminated by the coxal glands, whereas most of the K is eliminated by the anal system. Furthermore, the coxal fluid showed little change in K concentration with increased haemolymph K concentration during dehydration or increased dietary intake of K (Section II). The anal system of hydrated animals did, however, respond to both increased dietary Na and K intake (Section II), but did not show an increase in Na excretion in dehydrated animals in which the net ingestion of Na was increased due to reduced coxal Na excretion.

The close association of coxal excretion with feeding in *P. antipodiana* suggests that the coxal fluid is involved in the feeding process (Section II). However, irrespective of any involvement in feeding, the coxal glands also serve an excretory function. Under somewhat artificial conditions (extreme dietary and injected salt loading) the coxal glands were seen to function as excretory organs (Section II). Here under conditions which *P. antipodiana* is more likely to encounter (feeding during dehydration followed by a period of rehydration) the coxal glands once again demonstrated an excretory function. During dehydration the coxal glands respond to small increases in the osmotic pressure and Na concentration of the haemolymph with large increases in the osmotic pressure and Na concentrations of the coxal fluid. Consequently, although the rate of coxal excretion is reduced in dehydrated animals it remains a significant route for the excretion of Na. Furthermore during rehydration when the spiders eliminated the excess ions from the meal which they could not excrete during dehydration, the coxal glands again play a significant role in the elimination of Na. This involved the production of coxal fluid by animals which were not feeding.

Although the coxal glands do excrete at times other than feeding

much of the excretion of ions and water by the glands is anticipatory, excretion and ingestion occurring simultaneously. Anticipatory excretion is not unknown in the arthropods. Lockwood (1961) found that following the transfer from seawater to freshwater *Gammarus deubeni* formed hypo-tonic urine at blood concentrations up to twice that at which hypo-tonic urine is formed by animals fully adapted to freshwater, and Norfolk (1978) proposed an anticipatory regulation of urine production in the crab *Carcinus maenas*. Although the anticipatory excretion of *P. antipodiana* certainly involves the animal in a certain amount of risk, the degree of risk is minimised as excretion coincides with feeding, and under normal situations this would involve a guaranteed input of ions.

*P. antipodiana* was chosen for this study because it is a representative of the mygalomorph spiders, which are more generalised and ancestral to the araneomorphs, or true spiders (Comstock, 1940; Gerstch, 1979). In this sense *P. antipodiana* has served admirably, and now, I hope, that not only can many more questions be asked of *P. antipodiana* and the related mygalomorphs, but the osmoregulatory mechanisms that contribute to the success of the araneomorphs in osmotically more demanding environments can be interpreted with respect to this more generalised spider. It certainly would be interesting to consider the osmoregulatory physiology of spiders such as the Katipo, *Latrodectus katipo*, which is restricted to webs spun at the base of Marram grass or sedges in the sand dunes, or the spider *Desis marina* which lives below the high water mark in the holdfasts of *Durvillea antarctica*. Its only source of water other than its prey seawater.

## IV.5 SUMMARY

1. The effect of water deprivation and dehydration on the functioning of the anal system and coxal glands, and the ion and water balance of dehydrated *Porrhothele antipodiana* were studied.
2. Water deprivation resulted in a marked reduction in the volume of urine excreted by *P. antipodiana* following a meal. Periods of dehydration before feeding resulted in little further reduction in the urine volume excreted following a meal.
3. The rate of coxal excretion ( $\mu\text{l hr}^{-1} \text{ gm}^{-1} \text{ coxal opening}^{-1}$ ) during feeding was inversely related to the hydration state of the animals.
4. The stercoral fluid of water deprived feeding animals was iso-osmotic to the haemolymph, but as in hydrated animals, it was markedly hypo-ionic with respect to the ions Na, K and Cl.
5. The coxal fluid of dehydrated animals was iso-osmotic to the haemolymph. Na was the major cation present in the coxal fluid and in the coxal fluid of dehydrated animals its concentration was increased compared to that of hydrated animals. The K concentration showed little change with dehydration.
6. Reductions in the rates of anal and coxal excretion of dehydrated fed animals resulted in the spiders achieving a net gain of water from the prey which offset transpirational and excretory losses.
7. The inability of *P. antipodiana* to produce an excretory fluid that was more than iso-osmotic to the haemolymph limited the ability of the animals to excrete the ions ingested with the meal. Only 40% of the ingested Na and 13.2% of the ingested K were excreted by dehydrated fed spiders. Consequently the net effect of feeding was the ingestion of a fluid markedly hyper-osmotic to the haemolymph.
8. Spiders fed during dehydration excreted excess ions when free water was made available for rehydration.

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